AN ABSTRACT OF THE DISSERTATION OF

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Trichloroethene (TCE) is the most frequently detected organic contaminant in groundwater, is classified as a probable human carcinogen, and exhibits toxicological effects on the human endocrine, immune, developmental, and reproductive systems. While significant research efforts have been devoted to the development of strategies for remediating TCE-contaminated groundwater, their advancement is currently hindered by limitations in current methodologies for measuring in situ reductive dechlorination rates, especially for sorbing solutes. This dissertation describes the development, evaluation, and demonstration of a method for measuring in situ reductive dechlorination rates that utilizes single-well, "push-pull" test technology. Initial field tests indicated that trichlorofluoroethene (TCFE) could be used as a surrogate for TCE in push-pull tests since (a) TCE and TCFE were transported similarly and (b) TCFE underwent reductive dechlorination by a pathway analogous to that of TCE while retaining the fluorine label. Because TCFE and TCE experienced sorption at the selected field site, a novel data analysis technique called "forced mass balance" (FMB) was developed to obtain in situ transformation rates of sorbing solutes from push-pull test data. The FMB technique was evaluated by quantifying errors in rates derived by applying FMB to push-pull test data generated by a numerical model. Results from simulated tests indicated that an example in situ rate for the reductive dechlorination of TCFE, which was obtained by applying FMB to field data, was underestimated relative to the true in situ rate by 10%. The utility of the rate-determination method presented in this dissertation was demonstrated by using it to evaluate the effectiveness of a chemical amendment, namely fumarate, at enhancing in situ reductive dechlorination rates in TCE-contaminated groundwater. Reductive dechlorination rates increased following three consecutive additions of fumarate in all five of the tested wells.

The development of the rate-determination method described in this dissertation advances the state of bioremediation technology because methods for measuring in situ transformation rates are needed to both assess the potential for natural attenuation and to quantify the effects of bioremediation techniques in the field.

MEASURING IN SITU REDUCTIVE DECHLORINATION RATES IN TRICHLOROETHENE-CONTAMINATED GROUNDWATER

by

Kimberly J. Hageman

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CONTRIBUTION OF AUTHORS

Drs. Jennifer A. Field and Jonathan D. Istok provided guidance in all aspects of this dissertation. Dr. Lewis Semprini provided guidance in designing and interpreting results from field experiments and edited chapters 2 and 4. Dr. Timothy E. Buscheck facilitated the field campaigns and edited chapter 2. Dr. Martin H. Schroth provided guidance in designing and interpreting computer modeling experiments and edited chapter 3.

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CHAPTER 1. INTRODUCTION

Trichloroethene (TCE) is a synthetic chlorinated hydrocarbon known for its solvent properties and low fire and explosion potential. TCE has been used extensively since the 1920's for dry cleaning, degreasing fabricated metal parts, and as a solvent for fats, waxes, resins, oils, rubber, paints, and varnishes since the 1920's (*1*). Due to the common occurrence of leaks, spills, and poor disposal practices at industrial sites, significant volumes of TCE have been released to the environment. Released TCE tends to volatilize to the atmosphere since its Henry's Law constant and boiling point are 0.020 atm-m^3/mol at 20 °C and 88 °C, respectively. However, because TCE is water soluble (1.1 g/L at 20 °C) and it does not sorb strongly to soils, much of the TCE that does not volatilize migrates into groundwater (*2*). Because the density of TCE (1.46 g/mL at 20 °C) is greater than that of water, TCE tends to migrate deep beneath the water table, contaminating all groundwater that it contacts. TCE is the most frequently detected organic contaminant in groundwater (*3*) and the most frequently detected chemical at Superfund sites (*4*).

While TCE lowers the risk for fires and explosions at industrial sites and therefore saves human lives, another type of risk to human health is created by the contamination of potential drinking water sources with TCE (*5*). The primary public health concern associated with TCE is cancer induction through chronic exposure to low levels of TCE in drinking water (*1*). While TCE itself is not suspected to be carcinogenic, the products of TCE metabolism in mammals are blamed for its mutagenic effects (*5*). Besides carcinogenicity, TCE exhibits toxicological effects on the endocrine, immune, developmental, and reproductive systems (*1*). The maximum contaminant level (MCL), or the lowest concentration to which a contaminant can be removed from drinking water using current technology, for TCE is 5 ppb (*6*). However, the California EPA recommends that the state MCL be lowered to 0.8 ppb based on results from recent toxicological studies (*1*).

Widespread concern about the toxicological effects of TCE has driven research activity to focus on the development of strategies for remediating TCE-contaminated

groundwater. As recently as 1980, TCE was thought to be non-degradable in groundwater (*2, 7*) and pump-and-treat was considered the only viable remediation strategy (*8*). However, pump-and-treat, which involves pumping contaminated groundwater to the surface for treatment, has been generally ineffectual (*9-11*). Moreover, surface treatment processes, such as air stripping or carbon adsorption, simply transfer TCE to another medium instead of destroying it (*9*). In the mid-1980's, investigators began to report the occurrence of TCE transformation products in groundwater (*2*). These findings inspired a new research effort directed towards understanding possible mechanisms of TCE transformation in groundwater. Since then, several reviews have been published that describe potential TCE transformation mechanisms by both abiotic and biological processes (*2, 5, 8, 12, 13*).

The average oxidation state of the carbon atoms in TCE is positive due to the presence of the three electronegative chlorine atoms in the molecule. As a result, these carbon atoms do not have electrons available to donate, which makes it difficult for microorganisms to obtain energy from TCE via oxidation. For this reason, TCE is recalcitrant in aerobic aquifers relative to other common contaminants, from which microorganisms can gain energy by mediating the transfer of their available electrons to oxygen. Although no microorganism is known to exist that can use TCE as its sole electron source (*14, 15*), TCE can be oxidized by aerobic organisms through cometabolism (*2, 5, 14*). The aerobic cometabolism of TCE relies on the fortuitous oxidation of TCE by enzymes produced by microorganisms for other purposes. Concerted research efforts are underway to better understand the mechanisms that drive the transformation of TCE by aerobic cometabolism and to promote the transformation of TCE by aerobic cometabolism in remediation projects (*16-18*).

In anaerobic aquifer environments, the predominant pathway for TCE transformation is reductive dechlorination (*8, 13, 15, 19*). In this reaction, microorganisms gain energy by mediating the transfer of electrons from electron donors to TCE. In the reductive dechlorination pathway, hydrogen atoms replace chlorine atoms in the TCE molecule thereby driving the sequential reduction of TCE to the dichloroethene (DCE) isomers, chloroethene (CE), and ethene. Among the pure culture strains capable of facilitating the reductive dechlorination of TCE are *Dehalospirillum multivorans* (*20*), *Dehalococcoides ethenogenes* Strain 195 (*21*), *Desulfitobacterium* strain PCE-S (*22*), and *Dehalobacter restrictus (23)*. However, none of these pure cultures are capable of reducing TCE to the completely dechlorinated product, ethene, which is the desired end product of reductive dechlorination

due to its lack of toxicity. It is especially concerning when reductive dechlorination results in the accumulation of CE since CE is most toxic of the chemicals in the TCE reductive dechlorination pathway. However, microbial communities composed of syntrophically-associated microorganisms are capable of performing complete reductive dechlorination (*24-26*).

Based on the wealth of information that has been obtained about the reductive dechlorination pathway, in situ bioremediation via reductive dechlorination has become a predominant strategy for remediating TCE-contaminated groundwater (*27, 28*). The two primary management approaches associated with in situ bioremediation are "monitored intrinsic bioremediation" and "engineered bioremediation." Intrinsic bioremediation relies on indigenous microorganisms to reduce contaminant mass without human intervention. The natural attenuation of TCE by reductive dechlorination occurs when dechlorinating microbial communities co-exist with co-contaminants or naturally-occurring organic compounds that can act as electron donors (*29*). The use of intrinsic bioremediation as a management strategy requires proof that contaminant loss due to transformation is occurring (*30*). Additionally, intrinsic bioremediation protocols often require that computer modeling be used to predict the future migration and attenuation of contaminant plumes (*28*). To obtain accurate predictions, site-specific rates for in situ transformation must be obtained so that they can be used as input values in solute fate and transport models.

Where natural attenuation does not result in the complete conversion of TCE to ethene or where transformation rates are too slow to meet risk management goals, engineered bioremediation approaches are needed. A common engineered approach to enhancing in situ reductive dechlorination rates is to stimulate the growth of indigenous dechlorinating microorganisms by adding chemical amendments to groundwater. A wide variety of chemicals and chemical mixtures have been evaluated for their suitability as amendments for enhancing reductive dechlorination. Lee et al. reviewed results from laboratory tests that were designed to assess the effectiveness of potential amendments such as complex organic mixtures (molasses, wastewater, cheese whey permeate, corn steep liquor, manure tea), metabolic intermediates (benzoate, lactate, propionate, acetate, butyrate), alcohols (methanol, ethanol), molecular hydrogen, sulfate, nitrate, vitamins, and micronutrients (*29, 31*). While many of these amendments were effective, disadvantages were associated with each and none were universally effective. The effectiveness of a chemical amendment is generally evaluated

by comparing reductive dechlorination rates measured with and without the chemical amendment in laboratory experiments with pure or mixed cultures of microorganisms. However, due to potential discrepancies between laboratory and field results (*28, 32, 33*), it is also necessary to evaluate the effects of chemical amendments on in situ reductive dechlorination measured during field tests.

While protocols for remediating TCE-contaminated groundwater by intrinsic or engineered bioremediation require methods for measuring in situ transformation rates, in situ transformation rates are difficult to measure. In situ transformation rates are difficult to measure because solute concentrations in groundwater are affected by both transformation and transport processes (*27, 28, 34*). For example, solute concentrations measured at a single well change with time due to advection, which is the process by which solutes are transported with bulk groundwater flow. Dispersion is the process by which solute concentrations decrease due to the broadening of solute plumes as they flow through the porous aquifer medium. There are three basic processes that cause dispersion (*35*). First, when a fluid travels though pores, the velocity of the fluid is faster in the center of the pore than along the edges. Second, some fluid will follow longer flow paths than other fluid. Third, the velocity of fluid traveling through larger pores is greater than that flowing through smaller pores. Sorption is the process by which solutes partition to aquifer solids, thereby decreasing their aqueous concentrations and slowing their rate of transport in groundwater.

A number of in situ rate-determination methods have been described that involve the measurement of reactant and product concentrations in multiple wells. For example, McAllister and Chiang described the "mass balance approach" (*36*). In this approach, the total mass of a reactive solute (reactant) in an aquifer is determined by collecting groundwater samples from an extensive monitoring well network that encompasses the complete vertical and horizontal extent of the solute plume. In situ transformation rates are calculated from changes in the total mass of the solute over time. In the "Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater" published by the United States EPA in 1998, a "data set normalization" approach for determining in situ transformation rates was described (*28*). This approach relies on the presence of a nonreactive tracer that is associated with the contaminant plume. Example tracers include trimethylbenzene, which is a nonreactive component of fuel hydrocarbons; the chloride ion produced during reductive dechlorination; and the carbon nucleus of chlorinated ethenes. Reactant and tracer

concentrations are measured in multiple wells arranged along a transect. The effects of transport processes are accounted for by normalizing measured reactant concentrations to their corresponding measured tracer concentrations. Finally, Buscheck and Alcantar presented a rate-determination method that utilizes a novel analytical solution to the advection-dispersion equation (*37*). Reactant concentrations are measured in multiple wells arranged in a transect and then the analytical solution is used to determine the transformation rate that would be necessary to produce a steady-state plume of the configuration found at the field site. However, McNab and Dooher cautioned against the use of this method because it produces spurious results if the solute plume has not truly reached a steady-state condition (*38*).

Temporal differences in reactant and product concentrations measured at a single well can also be used to determine in situ transformation rates. Methods that use single wells tests are advantageous over those that require multiple wells for a number of reasons. For example, single-well tests are cost-effective relative to multi-well tests because fewer groundwater wells, which are expensive to construct, are needed. Additionally, single-well tests do not require the fortuitous association of a nonreactive tracer with the solute plume or that the solute plume has reached steady-state. Single-well tests take less time to conduct than multi-well tests since injected solutes do not have to be transported between wells. Because single-well tests are time-efficient, they can be repeated in a single well to assess reproducibility or to compare rates obtained with different chemical amendments. At sites where a number of monitoring wells exist, single-well tests can be conducted simultaneously in different wells to assess spatial variability. Washington et al. described a method that utilizes reactant concentrations measured at a single well and site-specific hydrologic properties with "full inverse" modeling to differentiate concentration changes due to transformation from those due to transport processes (*33*). This dissertation describes the development and demonstration of an alternative approach that uses data collected during single-well "push-pull" tests to determine in situ transformation rates. Push-pull tests are conducted by injecting ("pushing") an aqueous test solution containing a conservative tracer and one or more reactants into an aquifer via a groundwater well (*39*). Samples of the test solution/groundwater mixture are then extracted ("pulled") from the same well over time and analyzed for tracer, reactant, and product concentrations.

In chapter 2 of this dissertation, the transport and transformation behavior of trichlorofluoroethene (TCFE) in a TCE-contaminated aquifer is described. The TCE-

contaminated aquifer was located at the site of a former pesticide-manufacturing site in the San Francisco Bay area. Because field experiments described in chapter 2 indicated that the transport and transformation behavior of TCFE was similar to that of TCE, TCFE was used as a surrogate for TCE in proceeding push-pull tests. TCE itself was not used in push-pull tests because mixing of the injected test solution with native groundwater would have rendered it impossible to distinguish injected and background TCE. In chapter 3, a novel data analysis technique for determining in situ transformation rates of sorbing solutes from push-pull test data is described. While this data analysis technique can be used with a variety of sorbing solutes, its development was especially critical to this dissertation because TCFE and TCE sorb to aquifer materials at the selected field site. In chapter 4, the utility of the rate-determination method developed in chapters 2 and 3 is demonstrated by using it to quantify the effects of a chemical amendment, namely fumarate (*trans*-1,2-ethenedicarboxylate), on in situ transformation rates.

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CHAPTER 2. IN SITU ANAEROBIC TRANSFORMATION OF TRICHLOROFLUOROETHENE IN TRICHLOROETHENE-CONTAMINATED GROUNDWATER

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ABSTRACT

Methods are needed to obtain in situ information on the transformation rates of trichloroethene (TCE), the most commonly detected organic groundwater contaminant. The objective of this research was to investigate the potential for determining TCE transformation rates in groundwater by measuring the transformation rate of its fluorinated surrogate, trichlorofluoroethene (TCFE). To explore this hypothesis, the in situ transport behavior, transformation pathway, and transformation rate of injected TCFE were determined in TCE-contaminated groundwater using single-well, push-pull tests. Although transport behavior varied between wells, TCFE, dichlorofluoroethene (DCFE), and TCE were transported similarly to each other. In the shallow water-bearing zone, TCFE was reductively dechlorinated to *cis*-DCFE, *trans*-DCFE, and (*E*)-1-chloro-2-fluoroethene (CFE), while co-injected TCE was concurrently transformed to *cis*-dichloroethene (DCE), *trans*-DCE, 1,1-DCE, and a trace amount of chloroethene (CE). With added formate and the injected TCFE concentration being a factor of 20 higher than that of TCE, the TCFE transformation rate ranged from 0.053 to 0.30 μmol/L-day, while that of TCE ranged from 0.009 to 0.012 μmol/L-day. Without added formate, the TCFE transformation rate decreased to 0.036 μmol/L-day. In the deeper water-bearing zone, TCFE transformation occurred only after a lag time of 55 days with added formate. No TCFE transformation occurred in groundwater that had not previously exposed to TCE. The potential applicability for TCFE as an in situ transport and transformation surrogate for TCE was demonstrated.

INTRODUCTION

Trichloroethene (TCE), a non-flammable solvent used in large quantities by industry, is the most common organic groundwater contaminant (*1*) and is classified as a "probable human carcinogen" (*2*). Due to evidence that subsurface microorganisms are capable of degrading TCE under specific biogeochemical conditions, in situ bioremediation of TCE-contaminated groundwater is being investigated (*3*). TCE degradation under methanogenic and sulfate-reducing conditions in laboratory (*4*) and field studies (*5-10*) has been reported. Anaerobic TCE degradation occurs by reductive dechlorination, a reaction in which hydrogen atoms sequentially replace chlorine substituents. Thus, in the commonly observed TCE transformation pathway, TCE is sequentially reduced to the dichloroethene (DCE) isomers, chloroethene (CE), and ethene (Figure 2.1a).

In situ TCE transformation rates, which are needed to assess the potential for intrinsic bioremediation and to design and monitor engineered bioremediation projects, have been reported (*11*). However, the common method for estimating in situ rates, monitoring temporal

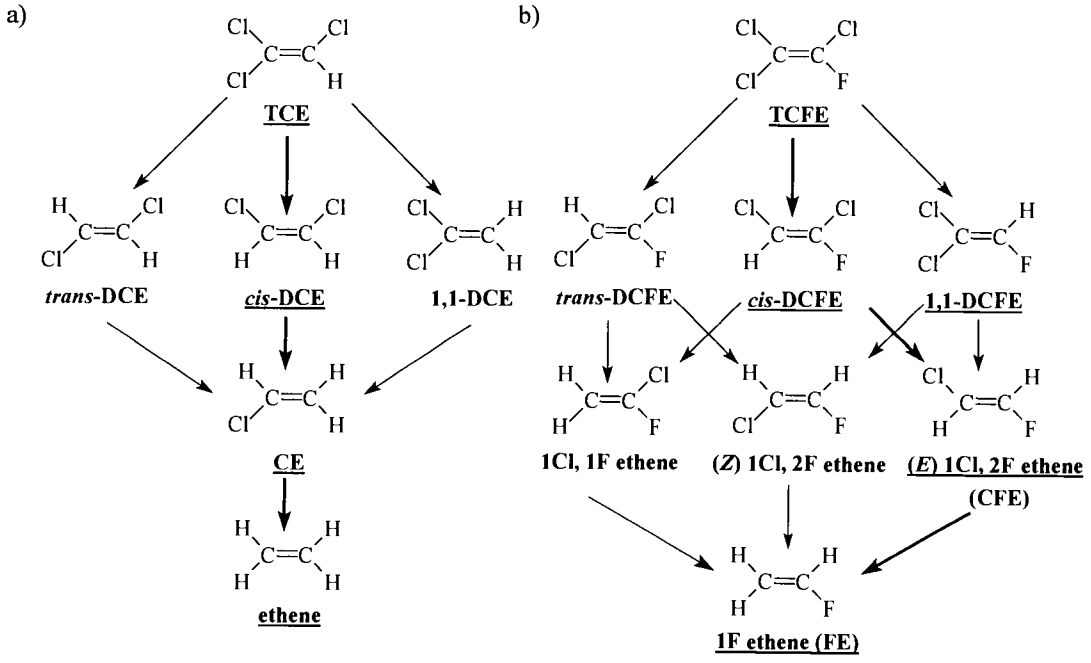

Figure 2.1. Reductive dechlorination pathways for (a) TCE (*5*) and (b) TCFE (*12*). The predominant isomers and pathways are indicated by underlines and heavy arrows.

and spatial changes in TCE and transformation product concentrations, is problematic. Vancheeswaran et al. (*12*) argued that transformation rates obtained in this way are often ambiguous because (a) small changes in TCE and transformation product concentrations are difficult to measure in the presence of high background concentrations, (b) microbially-generated transformation products cannot be distinguished from those that are present in the background, and (c) concentration changes due to transformation are obscured by non-biological processes such as advection, dispersion, sorption/desorption and the dissolution of non-aqueous phase TCE. Furthermore, groundwater tracer tests that involve the addition of TCE to re-injected groundwater is problematic even if the endogenous TCE and its degradation products are removed first (e.g., by air sparging). In these types of tracer tests, dilution of the tracer test solution with background groundwater renders it impossible to distinguish between injected and background TCE and its degradation products.

An alternative approach, in which the specified problems are avoided, involves measuring the transformation rate of injected trichlorofluoroethene (TCFE) in TCE-contaminated groundwater and estimating the TCE transformation rate from that of TCFE (*12*). In groundwater microcosm experiments, trichlorofluoroethene (TCFE) was reductively dechlorinated to "fluorine-labeled" transformation products by a pathway analogous to that of TCE (*12*) (Figure 2.1b). Moreover, with comparable initial TCFE and TCE concentrations, zero-order TCFE and TCE transformation rates were similar in single-compound tests as well as in tests where TCFE and TCE were present together. In free enzyme, corrinoid-mediated experiments (*13*) with comparable initial TCFE and TCE concentrations, reductive dechlorination of TCFE and TCE followed second-order kinetics and TCFE transformation rates were 12 to 25 times higher than those of TCE.

The objective of this research was to determine the transport behavior, transformation pathway, and transformation rate of TCFE under defined conditions in TCE-contaminated groundwater at a former chemical manufacturing plant in the San Francisco Bay area. To this end, single-well, push-pull tests with TCFE were conducted in two water-bearing zones with different contaminant and biogeochemical characteristics. In a "push-pull" test, a prepared test solution containing the compounds of interest and a conservative tracer is injected ("pushed") into the saturated zone of an aquifer and then extracted ("pulled") from the same location (*14, 15*). Breakthrough curves, which are used to assess the transport or

transformation behavior of the injected compounds, are constructed from samples collected during the extraction phase of the test.

EXPERIMENTAL SECTION

Chemicals

Trichloroethene (TCE) (99.5% purity), chloroethene (CE) (97%), sodium formate, and sodium bromide were obtained from Fisher Scientific (Fair Lawn, NJ). *Cis*-dichloroethene (*cis*-DCE) (97%), *trans*-dichloroethene (*trans*-DCE) (98%), and 1,1-dichloroethene (1,1-DCE) (99%) were obtained from Aldrich Chemical Company (Milwaukee, WI). Trichlorofluoroethene (TCFE) (97% pure, containing 0.1% *cis*-dichloroethene (DCFE) and 0.1% *trans*-DCFE) and DCFE (98% pure mixture consisting of 50% *cis* and 50% *trans* isomers) were obtained from ABCR Chemicals (Karlsruhe, Germany). 1,2-chlorofluoroethene (97% pure mixture consisting of 31% (*E*) and 69% (*Z*) isomers) was obtained from SynQuest Laboratories, Inc. (Alachua, FL). Fluoroethene (FE) (98%) was obtained from Lancaster Synthesis (Pelham, NH). Ethene (19.2 ppm in nitrogen) was obtained from Airco Special Gases (Vancouver, WA). 1-chloropropane and 1-chlorobutane, which were used as internal standards for gas chromatography (GC) quantitation, were obtained from Matheson Company (Cincinnati, OH) and Mallincrodt, Inc. (St. Louis, MO), respectively.

Site Description

The tests in this study were conducted in TCE-contaminated groundwater at a former chemical manufacturing plant in the San Francisco Bay area where TCE reductive dechlorination has been monitored in recent years (*7, 8*). Tests were conducted in two distinct water-bearing zones, the A-zone and the C-zone. The A-zone is an unconfined shallow layer composed mainly of placed fill over Bay Mud. The water table lies within 3 meters of the ground surface. The groundwater velocity ranges from 1.5 to 6 meters per year. The C-zone underlies the Bay Mud and is characterized by alluvial fan deposits, approximately 6 to 23 meters below the ground surface. Groundwater velocities range from 6 to 31 meters per year. The water table slopes to the west in both zones.

Monitoring well installations and subsurface investigations began at the site in the early 1980s. TCE and tetrachloroethene (PCE), pesticides, BTEX (benzene, toluene, ethylbenzene, and xylene), and metals were detected in the A-zone. TCE and PCE were detected in the C-zone. Neither DCE isomers nor CE were ever used or produced at the facility. Using a reductive dechlorination screening process that compares measured

concentrations of contaminants and biogeochemical indicators to threshold values (*16*), Buscheck found that there was strong evidence for reductive dechlorination in the A-zone and weaker evidence for reductive dechlorination in the C-zone (*8*).

Push-Pull Tests

A series of push-pull tests was conducted to obtain information on aqueous TCFE and TCE transport and transformation in the selected A-zone well, RI-10A, and in selected C-zone wells, GW-15C and GW-21C. These wells contain a range of background contaminant and

Table 2.1. Contaminants and Biogeochemical Indicators in Selected Wells

	Concentration (μmol/L)[a]		
	A-zone well	C-zone wells	
	RI-10A	GW-15C	GW-21C
trichloroethene (TCE)	ND[b]	240	ND
cis-1,2-dichloroethene (*cis*-DCE)	0.017	ND	ND
benzene	0.078	0.32	ND
toluene	0.0041	ND	ND
ethylbenzene	0.0029	ND	ND
total xylenes	0.005	ND	ND
ethene	ND	0.0043	ND
ethane	ND	ND	ND
methane	18	0.26	ND
total organic carbon	15,000	ND	ND
dissolved oxygen	130	5.6	18
nitrate-N	ND	ND	ND
sulfate	960	490	170
total dissolved iron	95	ND	ND

[a]Samples collected in May-June, 1999. Chlorinated hydrocarbons and BTEX compounds by EPA method 8021B; ethene, ethane, and methane by RSK-175; total organic carbon by EPA 9060; dissolved oxygen by membrane electrode probe; nitrate-N and sulfate by EPA 9056; iron by EPA 6010B. [b]Not detected.

biogeochemical indicator concentrations (Table 2.1).

Transport Tests. Test solutions consisted of tap water, bromide (to serve as a conservative tracer), TCFE and in one case, TCE (Table 2.2). Although it would have been desirable to use site groundwater in these experiments, the use of tap water was required to obtain regulatory approval to conduct these tests at this site. Note that because the purchased TCFE standard contained 0.1% *cis*-DCFE and 0.1% *trans*-DCFE, ~0.015 μmol/L of these compounds were also injected in every test. The test solution was prepared by adding bromide to the tap water and then sparging the solution for at least 4 hours with compressed air to mix and aerate the solution prior to injection. A concentrated aqueous solution of TCFE (and TCE, where applicable) was stored in a collapsible metallized-film gas-sampling bag (Chromatography Research Supplies, Addison, IL) to prevent volatilization losses during injection (Figure 2.2). TCFE and TCE were added to the tap water/bromide solution by metering the solution from the bag into the main injection line with a piston pump (Fluid Metering Inc., Oyster Bay, NY) (Figure 2.2). TCFE/TCE equilibration between the inner polyethylene layer of the bag and the TCFE/TCE solution was established by waiting at least 2 hours between filling the bag and starting the injection. TCFE/TCE equilibration between

Table 2.2. Push-Pull Test Descriptions

			Test Solution Composition			
test	**well**	**zone**	**bromide (mmol/L)**	**TCFE (μmol/L)**	**TCE (μmol/L)**	**formate (mmol/L)**
Transport Tests						
1	RI-10A	A	1.3	8.9	0.019	--
2	GW-21C	C	1.2	15	--	--
3	GW-15C	C	1.3	14	--	--
Transformation Tests						
4	RI-10A	A	1.2	16	0.78	2.0
5	RI-10A	A	1.3	13	--	--
6	GW-15C	C	1.3	19	--	8.3
7	GW-21C	C	1.4	33	--	--

the injection line tubing and the test solution was established by purging the injection lines with test solution for 10 minutes prior to starting the injection phase. Equilibration times were determined in preliminary laboratory experiments.

The injection/extraction procedure for tests in the A- and C-zones were not identical since well diameters were 2.5 cm in the A-zone and 10 cm in the C-zone. In the A-zone test, 50 L of test solution were injected into the bottom of the well at a flow rate of ~0.2 L/min (Figure 2.2a). In C-zone tests, ~250 L of test solution were injected between a pair of inflatable packers at a rate of ~2 L/min (Figure 2.2b). The packers were used to isolate a meter-long section of the well screen. In all tests, the test solution was injected through 6-mm nylon-braided tubing (Kuryama Co., Santa Fe Springs, CA) into the well with a Masterflex peristaltic pump (Barnant Co., Barrington, IL). Five to ten samples of the test solution, which

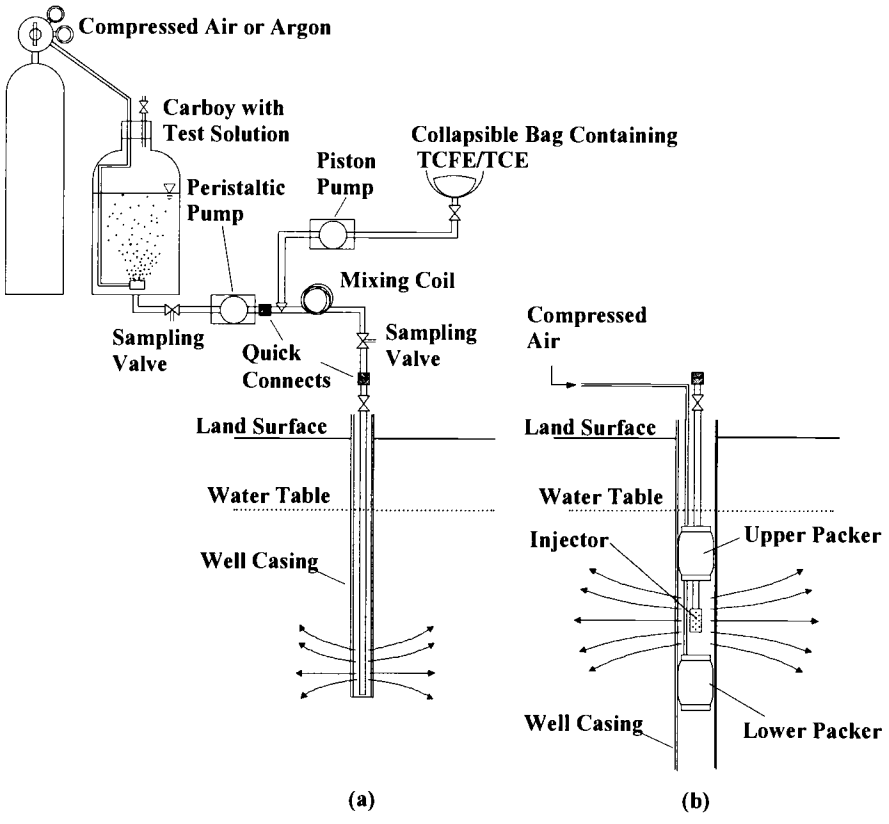

Figure 2.2. Experimental set-up for test solution injection in (a) the A-zone well and (b) C-zone wells (not drawn to scale).

were analyzed to determine injected concentrations, were collected from the sample valve during injection.

Immediately after completion of the injection phase, the carboy and the section of the injection line between the two quick connects were removed and the flow direction of the peristaltic pump was reversed. In the A-zone test, 67 L of test solution/groundwater mixture were extracted at a rate of ~0.2 L/min; 20 samples were collected. In C-zone tests, ~500 L of test solution/groundwater mixture were extracted at a rate of ~2 L/min; ~50 samples were collected. All samples were collected in volatile organic analysis vials without headspace, shipped on ice, and stored at 4 °C until analysis. Samples for volatiles were collected in duplicate and duplicate analyses were performed on approximately 10% of the samples. Samples for bromide were collected and analyzed in duplicate. All samples except those for bromide were preserved in 0.75% (v/v) concentrated HCl.

Transformation Tests. Two transformation tests were conducted in the A-zone well and one was conducted in each C-zone well. Test solutions consisted of tap water, bromide, TCFE, and in some cases, TCE and formate (Table 2.2). The test solution was prepared by adding bromide and formate to the tap water and then sparging the solution for at least 4 hours with compressed argon to mix and remove dissolved oxygen prior to the start of the injection phase. TCFE and TCE were then added to the test solution using a piston pump as described for the transport tests. The injected test solution volumes and injection flow rates for the transformation tests were identical to those described for the transport tests. Samples of the test solution/groundwater mixture were collected approximately once per week for up to 82 days. Prior to sample collection, A- and C-zone wells were purged by extracting 0.3 and 12 L of groundwater, respectively. Samples were preserved and stored as described for the transport tests.

Analytical Methods

Concentrations of TCFE, DCFE, CFE, TCE, DCE, and CE were determined by headspace analysis with gas chromatography/mass spectrometry (GC/MS). Qualitative analysis of FE and ethene were performed by solid-phase microextraction with GC/MS. The GC/MS system was composed of a Hewlett-Packard (Palo Alto, CA) model 5890 GC and 5972 series MS detector. Chromatographic separations were performed on a Supelco

(Bellefonte, PA) 30 m × 0.32 mm × 4 μm SPD-1 column. The identities of the TCFE degradation products were confirmed by comparing their spectra, which were obtained by operating the MS in scan mode, to published spectra (*12*). The MS was operated in selected ion monitoring mode for quantitation. 1-chloropropane and 1-chlorobutane were used as internal standards. The quantitation limits (signal/noise = 10) were ~0.005 μmol/L for analysis by headspace and ~0.2 μmol/L for analysis by solid-phase microextraction.

Bromide concentrations were determined by external calibration using a Dionex (Sunnyvale, CA) model DX-120 ion chromatograph equipped with an electrical conductivity detector and a Dionex AS14 column. Formate concentrations, as well as those of its potential degradation product, acetate, were determined by external calibration using a Waters Alliance (Milford, MA) high pressure liquid chromatograph (HPLC). The HPLC was equipped with a model 2690 separations module, a model 996 photodiode array detector, and a Phenomenex (Torrance, CA) 150 mm × 4.60 mm × 5 μm Luna C18 column.

RESULTS & DISCUSSION

Transport Tests

Transport tests were conducted to determine the relative transport behavior of injected TCFE, DCFE, and TCE in A- and C-zones prior to initiation of the transformation tests. The potential for anaerobic transformation of injected TCFE and TCE during transport tests was minimized by (a) air-saturating the test solution prior to injection and (b) completing each test in less than 10 hours. Transport test data were interpreted using breakthrough curves that display relative concentration against the cumulative volume extracted at the time the sample was collected divided by the volume of injected test solution. Relative concentration is defined as the measured concentration of a compound in a sample divided by the average concentration of the same compound in the injected test solution.

In the transport test conducted in RI-10A (test 1), the extraction phase breakthrough curves for TCFE and TCE were nearly identical (Figure 2.3a), indicating that TCFE and TCE were transported similarly. However, deviation of the TCFE/TCE breakthrough curve from that of bromide indicates that TCFE and TCE were not transported conservatively during the test. Since the test was conducted in less than 10 hours, we assume that the observed mass loss was not due to transformation but was instead caused by TCFE/TCE sorption to sediment

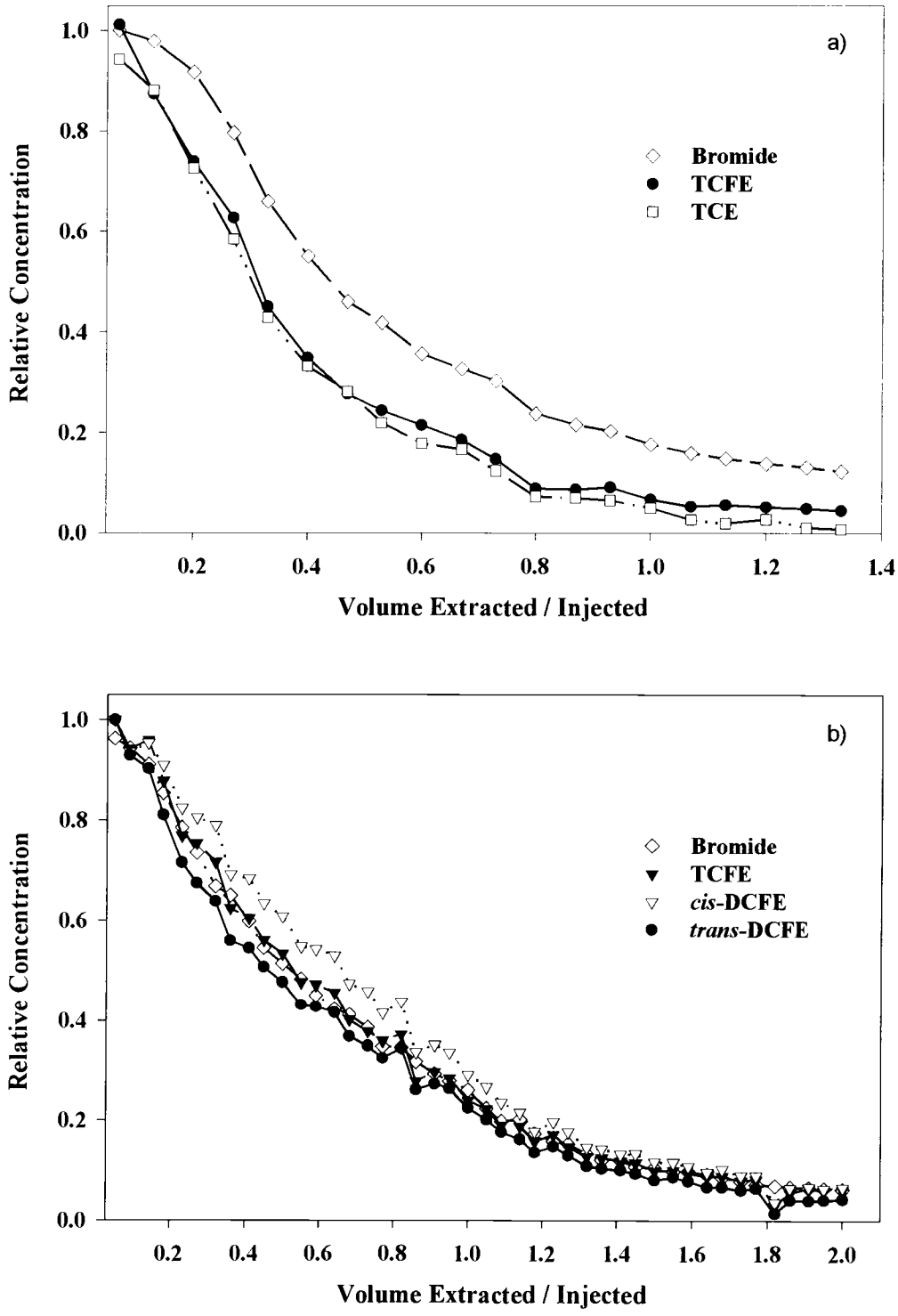

Figure 2.3. Breakthrough curves indicating (a) non-conservative transport of TCFE and TCE in A-zone well RI-10A (test 1) and (b) conservative transport of TCFE and DCFE isomers in C-zone well GW-21C (test 2).

organic matter and/or TCFE/TCE partitioning to nonaqueous phase liquids that may be present in the A-zone. However, no NAPLs were detected during construction of RI-10A. Mass balance calculations indicated that 58, 41, and 38% of injected bromide, TCFE, and TCE were extracted during the test, respectively. The similarity in TCFE and TCE transport behavior supports the hypothesis that TCFE can be used as a surrogate for TCE in TCE-contaminated groundwater. The transport behavior of *cis*- and *trans*-DCFE could not be determined in this test due to interference with residual *cis*- and *trans*-DCFE from a prior test conducted in this well.

The effects of injected solute sorption on push-pull test extraction phase breakthrough curves were studied in numerical simulations (*17, 18*). Based on those simulations, the TCFE and TCE transport behavior observed in test 1 cannot be attributed to equilibrium sorption with either linear or Langmuir isotherms. Thus, a more complicated sorption process, possibly influenced by diffusion-limited (nonequilibrium) mass transfer may be occurring.

In the transport test conducted in well GW-21C (test 2), TCFE, *cis*-DCFE, and *trans*-DCFE breakthrough curves were nearly identical to that of bromide (Figure 2.3b), indicating that all three compounds were conservatively transported. Mass balance calculations indicated that 69, 77, 64, and 70% of injected bromide, *cis*-DCFE, *trans*-DCFE, and TCFE were extracted during the test, respectively. TCFE was also conservatively transported in well GW-15C (test 3) (data not shown). The variation in transport behavior between wells may be due to varying soil organic carbon content or to the potential presence of NAPLs within the zone of influence of injected test solutions in some wells.. However, when the complete series of transport tests, which included two tests in the A-zone and four in the C-zone, is considered, no correlations between transport behavior and zone, background chlorinated ethene concentrations, or total organic carbon could be identified (data not shown).

Transformation Tests

Transformation tests were conducted (a) to determine if injected TCFE could undergo reductive dechlorination in the selected water-bearing zones, (b) to compare the in situ transformation pathways and rates for TCFE and TCE, and (c) to determine if reductive dechlorination of TCFE and TCE is limited by electron donor availability at this site.

A-Zone Tests. Test 4 was designed to test the hypothesis that the addition of formate would stimulate reductive dechlorination of TCFE and TCE in the A zone by serving directly as an electron donor or by stimulating other microbial activity that produce electron donors

that could be used by dechlorinating organisms. Relative concentrations for the bromide tracer (the measured bromide concentration C divided by the bromide concentration in the injected test solution, C_o) decreased with time as the test solution was gradually diluted with site groundwater (Figure 2.4a). By the end of the test, the bromide relative concentration was $C/C_o = 0.39$ indicating that this sample was a mixture of test solution (39 %) and groundwater (61 %). The effects of dilution on the concentration of a conservatively transported compound can be removed by dividing the compound's measured concentration by the relative concentration of the co-injected tracer (*19*). Conservative transport of formate was assumed for this study because of the high water solubility and negative charge of formate. Measured formate concentrations were divided by C/C_o to produce "dilution-adjusted" formate concentrations (Figure 2.5a and b). The rapid decrease in formate concentration suggests that an active anaerobic microbial community capable of utilizing formate was present. However, acetate, a potential fermentation product of formate resulting from acetogenesis, was not detected.

Measured TCFE concentration also decreased during test 4 (Figure 2.4b). The observed production of *cis*-DCFE, *trans*-DCFE, and (*E*)-1-chloro-2-fluoroethene (CFE) indicates that reductive dechlorination of injected TCFE occurred during this test. To increase the interpretability of the results and to compute transformation rates, it was necessary to adjust measured concentrations for dilution. However, the method used to adjust measured formate concentrations for dilution could not be employed since the transport test conducted in this well indicated non-conservative transport of TCFE, which means that relative concentrations of the bromide tracer cannot be used to correct for dilution of TCFE and its transformation products. Instead, an alternate dilution-adjustment method, which uses concentration ratios (*19*), was devised.

The method assumes that: (a) the transport behaviors of TCFE and its transformation products are identical, and (b) all potential TCFE transformation products are identified and quantified in each sample. The first assumption is supported by the observed identical transport behavior for TCFE and DCFE isomers in tests conducted in the A- and C-zone tests 1 and 3 (Figure 2.3) and by computed organic matter-water partition constants (K_{om}) for TCFE, *cis*-DCFE, *trans*-DCFE, CFE, and FE (log K_{om} = 2.7, 1.9, 1.9, 2.1, and 1.3, respectively). Values of K_{om} were computed from octanol-water partition constants (K_{ow}) (*20*), which were estimated from structural group contributions (*21*). For example, assuming

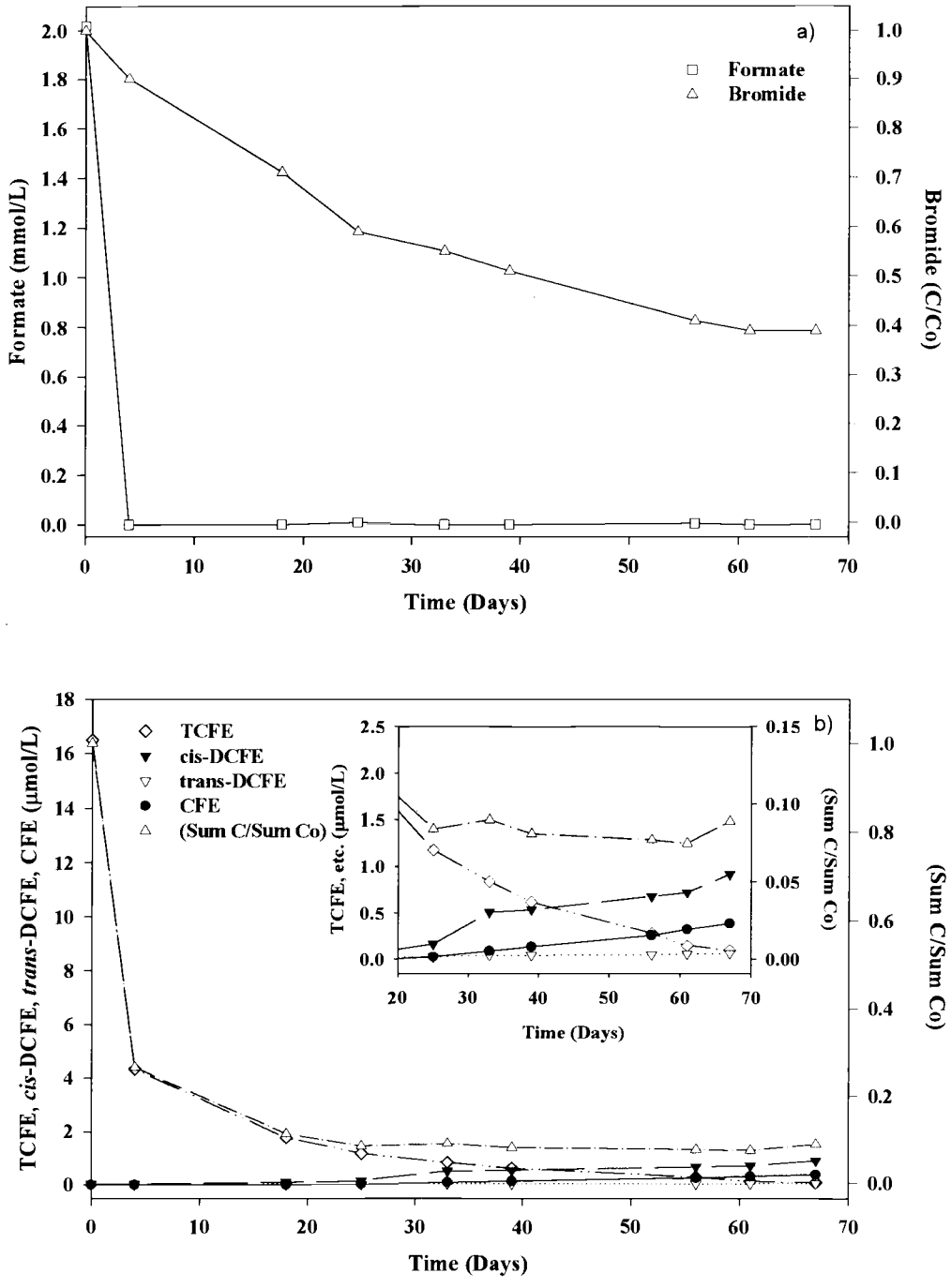

Figure 2.4. Test 4 data indicating (a) changes in concentration for formate and relative concentration for bromide and (b) reductive dechlorination of injected TCFE to *cis*-DCFE, *trans*-DCFE, and CFE in A-zone well RI-10A. Measured concentrations are not adjusted for dilution.

equilibrium linear sorption and estimated values for aquifer organic matter content in the A and C zones (f_{om} = 0.0006 - 0.03), bulk density (2.12 g/cm^3), and porosity (0.2), estimated retardation factors for TCFE and its transformation products ranged from 1 to 26.

The second assumption is considered valid because all currently known transformation products of TCFE and TCE (Figure 2.1) were analyzed by GC/MS. Although CO_2 and CH_4 production during reductive dechlorination of TCE in microcosm experiments has been observed by others, their production is thought to be the result of a combination of anaerobic chloroethene oxidation to acetate and acetotrophic methanogenesis (*22-24*). Since chlorofluoroethene was not produced until day 25 of the test, production of CO_2 and CH_4 from chlorofluoroethene is likely not responsible for the observed reduction in TCFE concentrations in test 4.

Measured concentrations of TCFE and its transformation products were corrected for dilution by dividing the measured concentration by the dilution factor ($SumC/SumC_o$) (Figure 2.4b) defined as

$$\frac{SumC}{SumC_o} = \frac{[TCFE] + [cis-DCFE] + [trans-DCFE] + [CFE]}{[TCFE]_o + [cis-DCFE]_o + [trans-DCFE]_o + [CFE]_o} \qquad (1)$$

where, for example, [TCFE] and $[TCFE]_0$ are the measured TCFE concentrations in a sample and in the injected test solution, respectively.

The dilution corrected concentrations for TCFE and its transformation products during test 4 are plotted in Figure 2.5a and for co-injected TCE and its transformation products during the same test in Figure 2.5b. Dilution adjustments for TCE and its transformation products were performed using an analogous equation to eq 1. Note that dilution-adjusted concentrations are displayed in Figure 2.5 and subsequent figures. The dilution-adjusted concentrations show the overall similarities in transformation pathways and rates for TCFE and TCE. TCFE transformation to *cis*-DCFE, *trans*-DCFE, and CFE occurred concomitantly with the transformation of TCE to *cis*-DCE, *trans*-DCE, and 1,1-DCE (Figure 2.5). The observed similarity in the in situ TCFE and TCE transformation pathways, including isomer predominance (Figure 2.1), indicates that it may be possible to use TCFE to determine the extent of TCE transformation, an important parameter in bioremediation design. Slower dechlorination between 0-18 days and faster transformation between 18-67 days, were similar for TCFE and TCE, which suggests that TCFE and TCE transformations were affected by similar rate-controlling factors. The TCFE concentration decreased at a nearly linear rate,

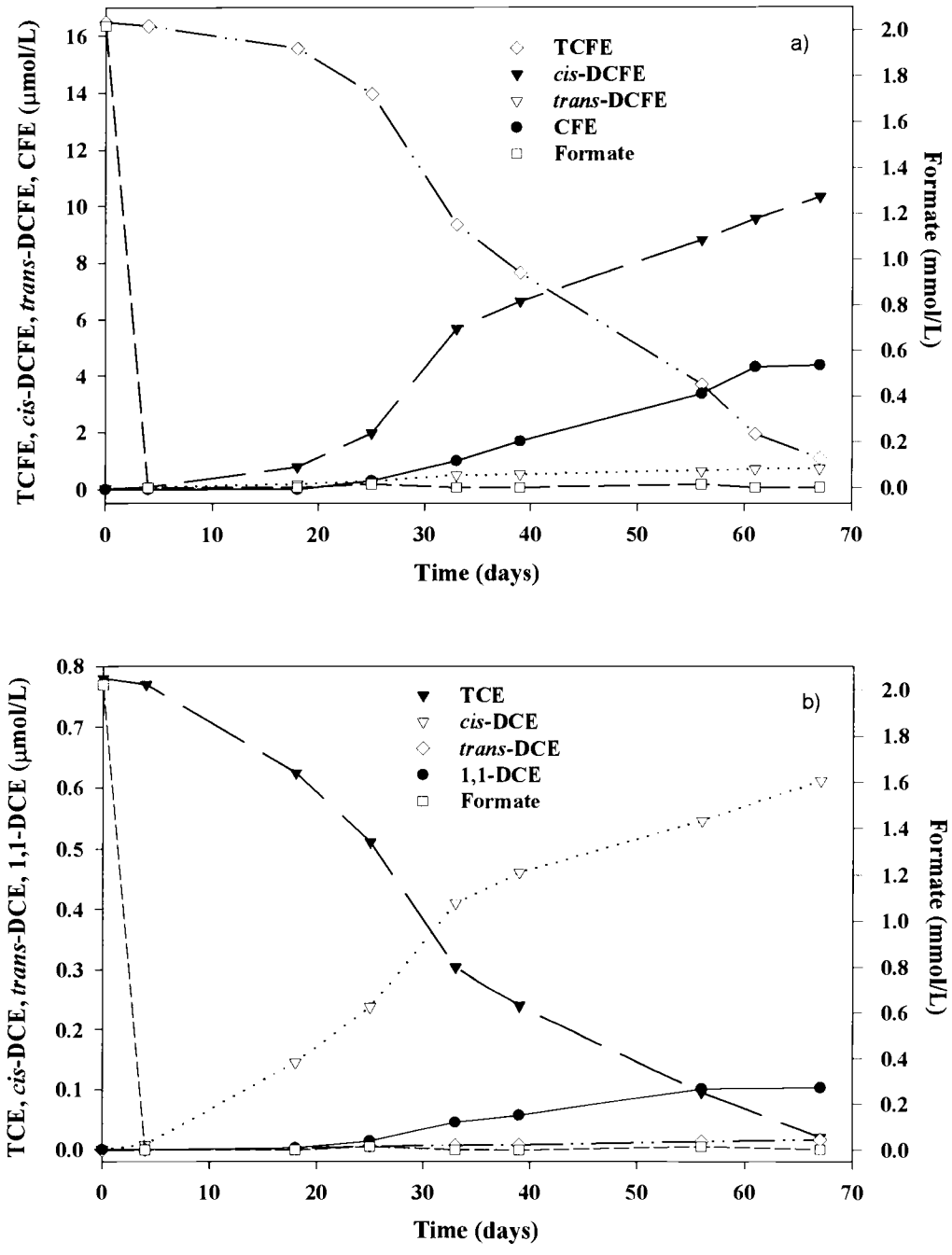

Figure 2.5. Reductive dechlorination of (a) injected TCFE to *cis*-DCFE, *trans*-DCFE, and CFE and (b) TCE to *cis*-DCE, *trans*-DCE, and 1,1-DCE in A-zone well RI-10A with added formate as an exogenous electron donor (test 4). Measured concentrations are adjusted for dilution.

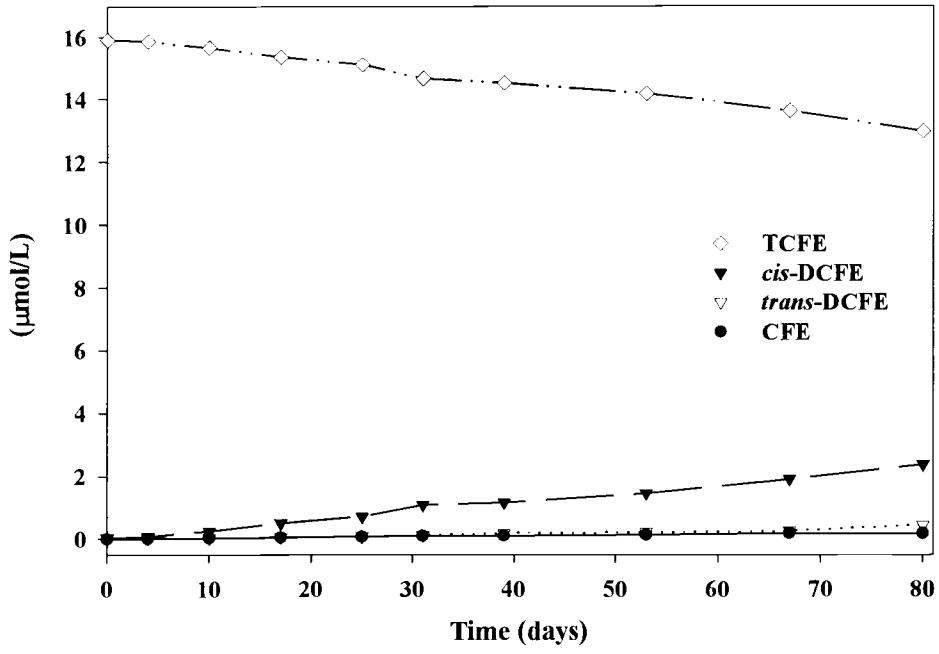

Figure 2.6. Reductive dechlorination of injected TCFE to *cis*-DCFE, *trans*-DCFE and CFE in A-zone well RI-10A without added formate as an exogenous electron donor (test 5). Measured concentrations are adjusted for dilution.

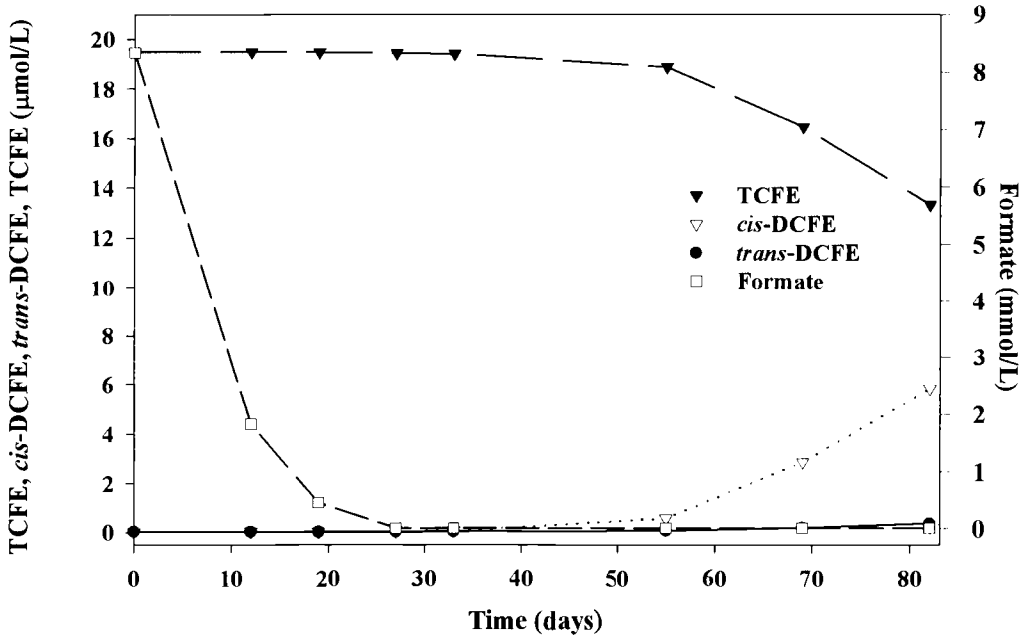

Figure 2.7. Reductive dechlorination of injected TCFE to *cis*- and *trans*-DCFE occurring after 55 days in C-zone well GW-15C with added formate as an exogenous electron donor (test 6). Measured concentrations are adjusted for dilution.

Table 2.3. Reductive Dechlorination Rates for TCFE, TCE, and Their Transformation Products in Push-Pull Transformation Tests

	Transformation Rates (μmol/L-day)*					
	A-zone tests			**C-zone tests**		
	Test 4: with formate		**Test 5: without formate**	**Test 6: with formate**		**Test 7: without formate**
days	**0-18**	**18-67**	**0-80**	**0-55**	**55-82**	**0-80**
TCFE	-0.053	-0.3	-0.036	0	-0.21	0
cis-DCFE	+0.044	+0.19	+0.029	0	+0.20	0
trans-DCFE	+0.009	+0.011	+0.0049	0	+0.011	0
CFE	0	+0.098	+0.0025	0	0	0
TCE	-0.009	-0.012	--	--	--	--
cis-DCE	+0.009	+0.009	--	--	--	--
trans-DCE	+0.00039	+0.00072	--	--	--	--
1,1-DCE	+0.00015	+0.0022	--	--	--	--

* "+" indicates increasing concentrations; "-" indicates decreasing concentrations

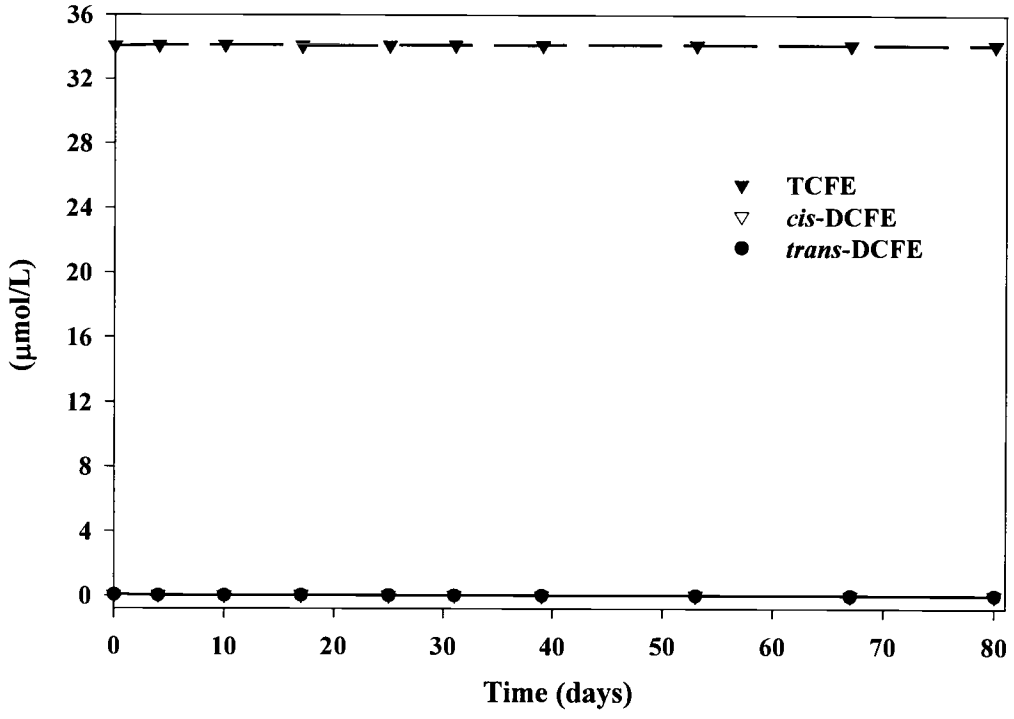

Figure 2.8. Absence of reductive dechlorination of injected TCFE in C-zone well GW-21C without added formate as an exogenous electron donor in groundwater that had not previously been exposed to TCE or other co-contaminants (test 7). Measured concentrations are adjusted for dilution.

which was determined by linear regression analysis to be 0.053 µmol-L between 0-18 days and 0.30 µmol/L-day between 18-67 days (Table 2.3). Ninety-three percent of the injected TCFE was transformed during the 67-day test. The TCE concentration decreased in a nearly linear manner at a rate of 0.009 µmol/L-day between 0-18 days and 0.012 µmol/L-day between 18-67 days (Table 2.3). Ninety-seven percent of the injected TCE was transformed to identified products during the test.

Although the overall percentages of TCFE and TCE transformation were similar; TCFE was transformed at a rate 5.8 times larger than TCE between 0-18 days and 25 times larger than TCE between 18-67 days. This rate difference is likely due to the higher injected TCFE concentration (20 times higher than TCE). TCE was injected at a lower concentration in this test to meet regulatory requirements.

Test 5 was designed to determine if TCFE transformation rates were limited by electron donor availability by conducting a second test in well RI-10A but without the addition of formate (Table 2.2). TCFE was again transformed to *cis*-DCFE, *trans*-DCFE, and CFE; FE was not detected (Figure 2.6). TCFE concentrations decreased and *cis*-DCFE, *trans*-DCFE, and CFE concentrations increased linearly. The TCFE transformation rate was 0.036 μmol/L-day and 19% of the injected TCFE was transformed during the 80-day test. These results indicate that TCFE transformation rates in well RI-10A were limited by the availability of electron donors since the transformation rate in test 5 without formate (Figure 2.6) was 1.5 to 8.3 times smaller than that in test 4 with formate (Figure 2.5). This observation is significant because it suggests that TCE transformation rates may be increased at this site by supplying exogenous electron donors.

C-zone Tests. Test 6, conducted in well GW-15C, was designed to determine the rate of TCFE transformation in the C-zone with formate added as an electron donor. Formate was utilized at a slower rate in this test compared to test 4 conducted in the A-zone, and was not completely degraded until day 27 (Figure 2.7). Acetate was not detected. No reductive dechlorination of TCFE was observed before day 55 (Figure 2.7). TCFE concentrations decreased at a rate of 0.21 μmol/L-day between 55-82 days (Table 2.3). Thirty-two percent of the injected TCFE was transformed to *cis*-DCFE and *trans*-DCFE during the 80-day test; CFE and FE were not detected. It was not possible within the scope of this project to conduct a second test in well GW-15C without formate.

Test 7, conducted in well GW-21C, was designed to determine if reductive dechlorination would occur in groundwater that had not previously been exposed to TCE or other co-contaminants (Table 2.1). Transformation of TCFE was not observed in well GW-21C (Figure 2.8), which is consistent with the hypothesis that dechlorinating microorganisms, if present at this location, would not be active in an uncontaminated portion of the aquifer. The observed slower rate of TCFE transformation in tests conducted in the C-zone compared to tests conducted in the A-zone is consistent with the conclusions drawn from the reductive dechlorination screening process conducted at this site by Buscheck (*8*). Although transformation was not observed in this well, TCFE was recovered during the 3-month long field experiment, which indicates that the push-pull test format is appropriate for this type of field application.

In summary, the potential applicability of TCFE as an in situ surrogate for TCE transport and transformation was demonstrated by the similarity in TCFE and TCE transport behavior, the composition of reductive dechlorination transformation products that formed in situ, and in the general agreement between the rates of transformation for TCFE and TCE.

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CHAPTER 3. "FORCED MASS BALANCE" TECHNIQUE FOR ESTIMATING IN SITU TRANSFORMATION RATES OF SORBING SOLUTES IN GROUNDWATER

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ABSTRACT

A method for estimating in situ transformation rates of sorbing solutes in groundwater is presented. The method utilizes a novel data processing technique called "forced mass balance" (FMB) to remove the effects of transport processes from reactant concentrations measured during single-well, "push-pull" tests. The effectiveness of the FMB technique was evaluated by quantifying errors in rates derived by applying FMB to push-pull test data generated by a numerical model. Results from simulated tests indicated that errors in derived rates increase as the test duration, groundwater velocity, and ratio of reactant to product retardation factors increase. In addition, errors in derived rates increase as the reaction rate constant and aquifer dispersivity decrease. As a demonstration, the FMB technique was used to derive an in situ reductive dechlorination rate for trichlorofluoroethene (TCFE) using data from a field push-pull test. Error analyses indicated that the in situ TCFE transformation rate was underestimated by a factor of 1.1 to 2. Thus, the FMB technique makes it possible to estimate in situ transformation rates of sorbing solutes and when FMB is coupled with computer modeling, errors in derived in situ rates can be quantified.

INTRODUCTION

Protocols for intrinsic and engineered approaches to groundwater remediation include steps for determining in situ transformation rates. However, in situ transformation rates are difficult to determine because reactive solute (reactant) concentrations in groundwater are affected by a combination of transformation and transport (advection, dispersion, sorption) processes. A number of methods use spatial differences in reactant and product concentrations to determine in situ transformation rates (*1-5*). Spatial differences are determined by collecting data from multiple wells arranged in transects or grids. However, wells are expensive and time-consuming to construct and some of these methods are limited to use with steady-state solute plumes (*3, 5, 6*) or plumes containing a nonreactive solute that can serve as a tracer (*3, 4*).

Temporal differences in reactant and product concentrations measured at a single well can also be used to determine in situ transformation rates. Single-well methods are advantageous in that only one well is needed per test, they are not limited to plumes with special characteristics, and site-scale variability can be assessed by comparing results from different wells at a single site. One approach is to measure reactant concentrations at a single well over time and then use site-specific hydrologic properties in combination with "full inverse" modeling to differentiate changes in reactant concentrations due to transformation from those due to transport processes (*7*). An alternative approach involves determining in situ transformation rates from data collected during single-well "push-pull" tests. Push-pull tests are conducted by injecting ("pushing") an aqueous test solution containing a nonsorbing, nonreactive tracer and one or more reactants into an aquifer via a monitoring well (*8*). Samples of the test solution/groundwater mixture are then extracted ("pulled") from the same well over time and analyzed for tracer, reactant, and product concentrations.

The in situ transformation rate of the reactant is then determined using a data processing technique to remove the effects of transport processes from measured concentrations of the reactant. In situ transformation rates were determined for injected reactants, including oxygen (*9*), nitrate (*9, 10*), sulfate (*11, 12*), acetate (*10*), and formate (*13*), from push-pull test data using a data processing technique that is hereafter referred to as "tracer-normalization." With this technique, the concentration of a nonsorbing reactant measured in an extraction sample is adjusted by dividing it by the relative concentration of the co-injected tracer measured in the same sample (*14, 15*). Thus,

$$[A]_{TN} = \frac{[A]}{[T]/[T]_o} \quad (1)$$

where $[A]_{TN}$ is the tracer normalized concentration of the nonsorbing solute, A; $[A]$ and $[T]$ are measured concentrations of A and the co-injected tracer, T, respectively; and $[T]_o$ is the injected concentration of T. Since the concentration of the tracer is affected by transport but not by transformation, the effect of transport processes on measured concentrations is expressed quantitatively by $[T]/[T]_o$. The validity of the tracer-normalization technique was verified by simulating push-pull tests with a modeling program and then comparing the rate constants derived from tracer-normalized concentrations to the rate constants used as inputs in the simulation program (*14, 15*).

However, the tracer-normalization technique was designed for use with nonsorbing reactants only (*14, 15*). Based on the desire to determine in situ reductive dechlorination rates of the sorbing reactant, trichlorofluoroethene (TCFE), Hageman et al. designed a data processing technique to remove the effects of transport processes (including sorption) from TCFE concentrations measured during push-pull tests (*13*). TCFE, which is sequentially transformed by reductive dechlorination to dichlorofluoroethene (DCFE), chlorofluoroethene (CFE), and fluoroethene (FE) in anaerobic environments, is of interest because it can be used as a surrogate for the priority pollutant, trichloroethene (TCE) (*13, 16*). The data processing technique presented by Hageman et al. is based on the assumption that TCFE and all of its products experience linear equilibrium partitioning and are transported identically to each other, but differently from the tracer.

Adjusted TCFE concentrations, from which in situ TCFE transformation rates are obtained, are calculated from

$$[TCFE]_{adjusted} = \frac{[TCFE]}{\Sigma/\Sigma_o} \quad (2)$$

where $[TCFE]$ is the measured aqueous concentration of TCFE in an extraction sample and Σ/Σ_o is an adjustment factor. The adjustment factor is calculated for each extraction sample from

$$\Sigma/\Sigma_o = \frac{[TCFE]+[DCFE]+[CFE]+[FE]}{[TCFE]_o+[DCFE]_o+[CFE]_o+[FE]_o} \quad (3)$$

where, for example, $[DCFE]$ and $[DCFE]_o$ are the measured aqueous concentrations of DCFE in an extraction sample and in the injected test solution, respectively. Since the sum of the

concentrations of TCFE and all of its products is affected by transport but not by transformation, the effects of transport processes on measured concentrations are expressed quantitatively by Σ/Σ_o. Note that this data processing technique requires quantification of all potential transformation products of TCFE. FE was not included in the equation defining Σ/Σ_o in Hageman et al. because it was not detected during field tests; however, it is included here for completeness. Adjusted concentrations for each transformation product are calculated with equations analogous to eq 2. Adjusted product concentrations are used to assess product distribution and the extent of dechlorination; however, transformation rates of intermediate products are not readily determined since they are concurrently being produced and transformed.

The objective of this work was to present and evaluate a data processing technique for use in determining in situ transformation rates of sorbing reactants whose products are not necessarily transported identically to the reactant or to each other. The technique, called "forced mass balance" (FMB), differs from that previously presented by Hageman et al. (*13*) in that the effects of transport processes (including sorption) are removed from total (aqueous plus sorbed) concentrations instead of just aqueous concentrations. Thus, for the reaction in which A is sequentially transformed to B and C, FMB-adjusted concentrations of the sorbing reactant, A, are calculated from

$$[A]_{FMB} = \frac{[A]_{aq+s}}{\Sigma_{aq+s}/\Sigma_{aq+s,o}} \tag{4}$$

where $[A]_{aq+s}$ is the total concentration of A associated with an extraction sample. The adjustment factor, $\Sigma_{aq+s}/\Sigma_{aq+s,o}$, is calculated for each extraction from

$$\Sigma_{aq+s}/\Sigma_{aq+s,o} = \frac{[A]_{aq+s}+[B]_{aq+s}+[C]_{aq+s}}{[A]_{aq+s,o}+[B]_{aq+s,o}+[C]_{aq+s,o}} \tag{5}$$

where, for example, $[B]_{aq+s}$ and $[B]_{aq+s,o}$ are the total concentrations of B in an extraction sample and in the injected test solution, respectively. The total concentration of A, $[A]_{aq+s}$, for example, is calculated from

$$[A]_{aq+s} = \frac{A_{aq}+A_s}{V_{aq}+V_s} \tag{6}$$

where A_{aq} is the mass of A in the extraction sample of volume, V_{aq}, and A_s is the mass of A in the volume of aquifer sediments, V_s, associated with the extraction sample based on the aquifer's porosity, n. The sorbed mass of A, A_s, for example, is calculated from

$$A_s = [A]_{aq} * K_{om} * f_{om} * V_s * \rho_g \quad (7)$$

where K_{om} is the organic matter/water distribution constant, f_{om} is the fraction of organic matter in aquifer sediments, and ρ_g is the grain density of aquifer sediments. FMB-adjusted concentrations for each transformation product are calculated with analogous equations. "Forced mass balance" is an illustrative term for this technique because the sum of FMB-adjusted reactant and product concentrations is conserved. Note that the aqueous reactant and product concentrations adjusted by the Hageman et al. method were also conserved (*13*).

To evaluate the FMB data processing technique, push-pull tests were simulated using a numerical model. FMB-adjusted concentrations were calculated from the simulated data and rate constants were derived from FMB-adjusted concentrations. Errors in derived rate constants were quantified with respect to reactant and product retardation factors, test duration, groundwater velocity, first-order rate constant, and dispersivity by comparing derived rate constants to the rate constants used as inputs in the numerical model. The FMB technique was then applied to data obtained during an actual field push-pull test designed to measure the in situ reductive dechlorination rate of trichlorofluoroethene (TCFE) in TCE-contaminated groundwater.

METHODS

Numerical Simulations

Push-pull tests were simulated with the fully implicit, volume-integrated, finite difference simulator, Subsurface Transport over Multiple Phases (STOMP) (*17*), with the one-dimensional linear form of the advection-dispersion equation. The mesh consisted of 400 equally spaced grid blocks (0.05 m × 1 m × 1 m). Bulk density, ρ_b, (2.3 kg/L) and porosity, n, (0.2) values were selected based on measurements conducted on aquifer materials collected at the field site where the actual push-pull test was conducted (see field push-pull test section). The groundwater (pore water) velocity, which was selected to represent that measured at the actual field site, was 0.01 m/day except where otherwise specified. The groundwater velocity was established by setting constant pressure boundaries at nodes 1 and 400. The dispersivity,

which was selected to represent that estimated by fitting modeled data to field data from the site, was 0.1 m except where otherwise specified.

The simulated test solution was injected into node 200 for 125 min at 2 L/min to replicate the injection procedure used during the field push-pull test. Simulated test solutions contained the sorbing reactant, A, which was allowed to undergo transformation by first-order kinetics in the aqueous phase only to the product, B. The input value for the first-order rate constant, k, was 0.069 day^{-1} except where otherwise specified and was selected because it was similar to that observed in the field test. The time step used during the injection phase was 0.05 min. So that the transport behaviors of the sorbing solutes could be compared to that of a tracer, simulated test solutions also contained the nonsorbing, nonreactive tracer, T. Following the injection, solutes were allowed to undergo transport and transformation for 90 days except where otherwise specified. Time steps were 0.05 days during this phase. To simulate sampling of the test solution/groundwater mixture, aqueous concentrations at the well (node 200) were output once per day. Although concentration versus distance data are not available during an actual field push-pull test, it was output at given times during simulated push-pull tests to aid in understanding the effects of transport behavior on concentrations at the single well. An example input file is included in the Supporting Information.

Test set I was designed to determine the accuracy in rates derived using FMB-adjusted concentrations for three illustrative cases. The three simulations were conducted using a retardation factor for A, *R(A)*, of 5. The retardation factor for B, *R(B)*, was 5, 1.25, and 20 during the three respective tests. Thus, *R(A)* was equal to *R(B)* in the first simulation, four times greater than *R(B)* in the second simulation, and four times less than *R(B)* in the third simulation. Test set II was designed to determine the effects of test duration, groundwater velocity, dispersivity, and input k on the accuracy of derived rates. For each of these parameters, a series of push-pull tests was simulated in which that single parameter was varied while the others were held constant. Simulations were conducted with *R(A)* and *R(B)* set to 5 and 1.25, respectively, and then with *R(A)* and *R(B)* set to 5 and 20, respectively. Test set III was designed to determine the effects of retardation factors on the accuracy of derived rates for a broader range of *R(A)* and *R(B)* combinations than that used in test set I.

Application of FMB to a Field Push-Pull Test

A field push-pull test was conducted in TCE-contaminated groundwater located at the site of a former chemical manufacturing plant in the San Francisco Bay area where TCE

reductive dechlorination has been monitored in recent years (*13, 18*). The test was conducted in the C-zone, which is characterized by alluvial fan deposits and is located approximately 6 to 23 m below the ground surface. C-zone groundwater velocities range from 6 to 31 m/year. The test solution (~250 L) was injected at a rate of ~2 L/min between a pair of inflatable packers designed to isolate a 1-meter section of the well screen. Extraction samples were collected once per week for 84 days. Further details about the field site, experimental design, and analytical methods are provided elsewhere (*13*).

A modeling exercise was used to quantitatively evaluate the accuracy of the rate derived from field data using the FMB technique. A series of simulations was conducted using the same input parameters used in test sets I-III. However, the injected test solution contained TCFE instead of the generic sorbing reactant, A. TCFE was allowed to undergo sequential transformation to DCFE and CFE (but not to FE since FE was not detected in extraction samples) by first-order kinetics in the aqueous phase only. Simulations were conducted with a range of input k values that bracketed the field-derived k value. Retardation factors of 2.05, 1.39, and 1.14 were used for TCFE, DCFE, and CFE, respectively, based on calculations using

$$R = 1 + \frac{K_{om} * f_{om} * \rho_b}{n} \tag{8}$$

where K_{om} is the organic matter/water partition coefficient of the solute; f_{om} and ρ_b are the fraction of organic matter and bulk density of aquifer sediments, respectively; and n is the porosity of the aquifer. The Estimations Programs Interface Suite (*19*) was used to assign K_{om} values of 90.5, 33.5, and 12.2 L/kg to TCFE, DCFE, and CFE, respectively. Values used for f_{om} (0.001), ρ_b (2.3 kg/L), and n (0.2) were selected based on measurements conducted on aquifer material collected at the field site. An input k value of 0.0017 day^{-1} was selected to describe the transformation of DCFE to CFE based on a best fit analysis between modeled and field data.

RESULTS AND DISCUSSION

Test Set I

Test 1: R(A) = R(B) = 5. At the end of the simulated test solution injection (time = 125 min), the maximum total concentrations of the co-injected conservative tracer, T, and the sorbing reactant, A, were at the well (distance = 0 meters) (Figure 3.1a). The concentration

profile of A was narrower than that of T because it sorbed to aquifer sediments during the injection while T did not sorb. After 90 days of transport and transformation, peaks of A and its product, B (formed in situ), were centered at an equal distance from the well (Figure 3.1b) but were chromatographically separated from the peak of T. This separation occurred because the transport velocities of A and B were less than that of T. The transport velocity of a solute equals the groundwater velocity divided by the solute's retardation factor. (Note that the retardation factor of T is 1.) The k used as an input in the simulation code for the transformation of A to B could have been back calculated by integrating total concentrations of A and B in the aquifer at any time. However, because concentration measurements are made at the single well location only during an actual field push-pull test, the challenge was to derive k exclusively from the total concentrations found at the single simulated well (Figure 3.1c).

FMB-adjusted concentrations of A and B were calculated from their total

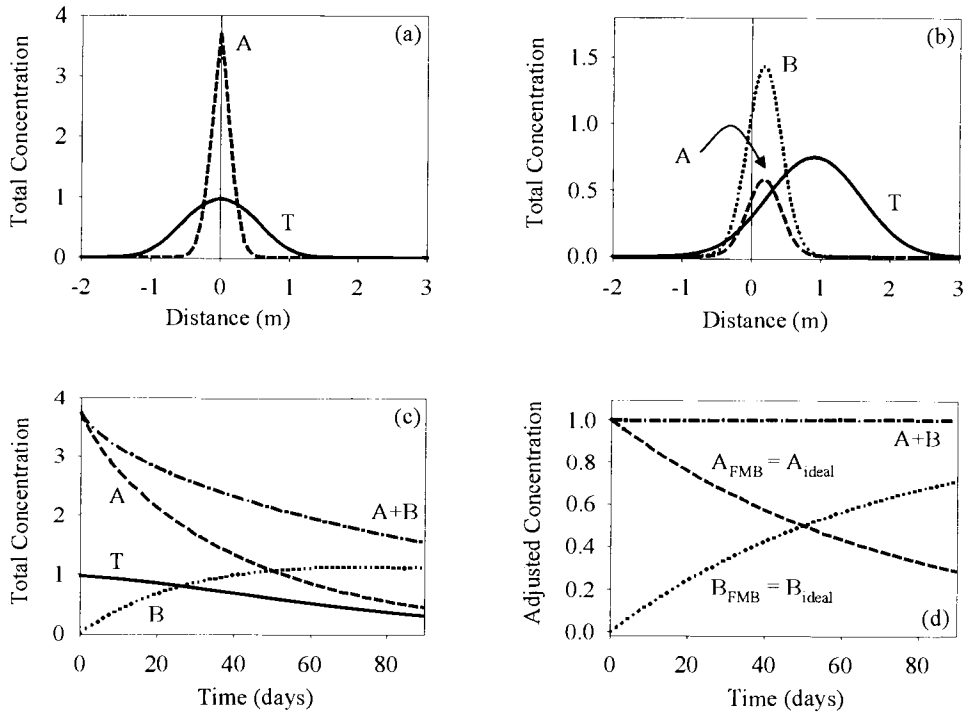

Figure 3.1: Test Set I, Test 1, *R(A)* = *R(B)* = 5. Total (aqueous plus sorbed) concentrations of the reactant, A, the product, B, and the tracer, T, in the simulated aquifer at (a) the end of the test solution injection (125 min) and (b) 90 days. The vertical line (at distance = 0 days) represents the single well. (c) Total concentrations and (d) FMB- and ideal transport-adjusted concentrations at the simulated well.

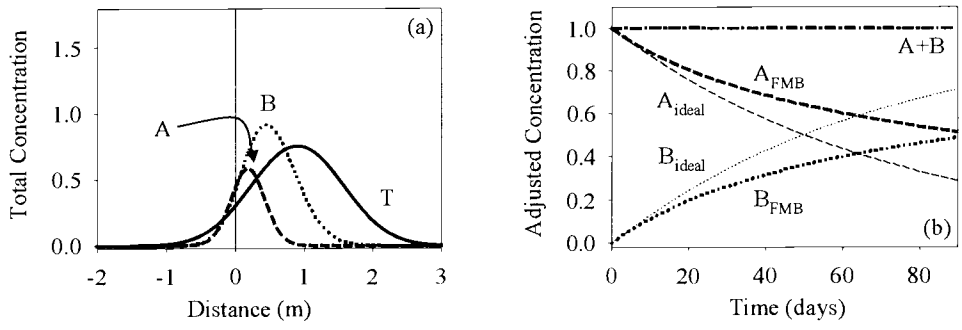

Figure 3.2: Test Set I, Test 2, *R(A)* = 5, *R(B)* = 1.25. (a) Total concentrations of A, B, and T in the simulated aquifer at 90 days. (b) FMB- and ideal transport-adjusted concentrations at the simulated well.

concentrations using eq 4 (Figure 3.1d). Ideally, the FMB technique removes the effects of transport processes (including sorption) from total concentrations. To test the effectiveness of the FMB technique, "ideal" transport-adjusted concentrations of A and B were calculated (Figure 3.1d). Ideal concentrations are those that would occur if the reaction were taking place in the absence of transport processes (e.g. in a batch reactor). Ideal (batch reactor) concentrations of A and B were calculated at each time point used in the simulation by

$$[A]_{ideal}^{t_n} = [A]_{ideal}^{t_{n-1}} - [A]_{ideal}^{t_{n-1}} * (t_n - t_{n-1}) * k / R(A) \qquad (9)$$

where $[A]_{ideal}^{t_n}$ is the ideal concentration of A at time, t_n. The value for k used in eq 9 was equal to the input k used in the simulations. It was divided by *R(A)* because when transformation is only permitted in the aqueous phase, the effective rate constant equals k divided by the retardation factor of the reactant. Ideal transport-adjusted concentrations of B were calculated at each time point as the difference between the initial ideal concentration of A and the ideal concentration of A at t_n. FMB- and ideal transport-adjusted concentrations of A and B were identical during this test (Figure 3.1d), indicating that the FMB technique successfully removed the effects of transport processes from total concentrations.

The performance of the FMB technique was also evaluated by comparing the k derived from FMB-adjusted concentrations to the k used as an input in the simulation model. The best fit slope of ln ($[A]_{FMB}/[A]_{FMB,o}$) versus time (data not shown), where $[A]_{FMB,o}$ is the injected FMB-concentration of A, represents $k/R(A)$ and was equal to 0.014 day^{-1}. Thus, the derived k (0.069 day^{-1}) was obtained by multiplying the slope by *R(A)* and was equal to the input k (0.069 day^{-1}). The error quotient, or the ratio relating the input and derived values for

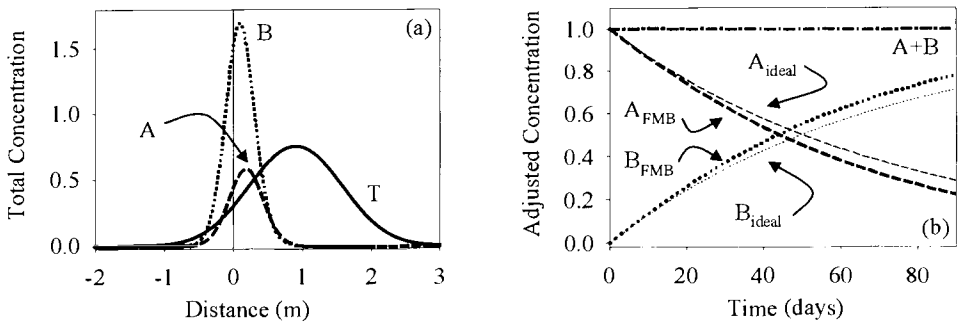

Figure 3.3: Test Set I, Test 3, *R(A)* = 5, *R(B)* = 20. (a) Total concentrations of A, B, and T in the simulated aquifer at 90 days. (b) FMB- and ideal transport-adjusted concentrations at the simulated well.

k, was 1. The conclusion that the FMB technique can be used to determine accurate in situ transformation rates when the reactant and product are transported identically was further supported by results from other simulated tests in which values of *R(A)* and *R(B)* were equal (data not shown).

Test 2: R(A) = 5, R(B) = 1.25. The concentration profiles of T and A at the end of the test 2 injection phase (data not shown) were identical to those observed at the end of the test 1 injection phase (Figure 3.1a). Furthermore, the peak areas of A and B at 90 days (Figure 3.2a) were identical to those observed at 90 days during test 1 (Figure 3.1b) because the effective rate constant, *k/R(A)*, was identical during the two tests. However, in test 2, peaks for A, B, and T were chromatographically separated from each other since each solute had a unique transport velocity. Because B was transported further downgradient during test 2 than during test 1, its total concentration at the well at 90 days was lower in test 2 than in test 1. As a result, the ratio of the total concentrations of A to B at the well at 90 days was higher in test 2 than in test 1. Since this ratio is proportional to the FMB-adjusted concentration of A (eqs 4-5), FMB-adjusted concentrations of A were higher in test 2 (Figure 3.2b) than in test 1 (Figure 3.1d) and deviated from their ideal transport-adjusted concentrations (Figure 3.2b). Likewise, FMB-adjusted concentrations of B were lower in test 2 than in test 1 and also deviated from their ideal concentrations. The deviation of FMB-adjusted concentrations of A from ideal concentrations caused the *k* derived from FMB-adjusted concentrations (0.041 day^{-1}) to be low relative to the input *k* (0.069 day^{-1}). Thus, the error quotient, or the ratio of the input *k* to the derived *k*, was 1.7. Results from this simulated test indicate that in situ transformation rates

estimated from field test data may be low by a factor of less than two when field conditions are similar to those used in this simulation.

Test 3: R(A) = 5, R(B) = 20. The concentration profiles of T and A at the end of the test 3 injection phase (data not shown) were identical to those at the end of the test 1 injection phase (Figure 3.1a). Furthermore, the peak areas of A and B at 90 days (Figure 3.3a) were identical to those observed at 90 days during test 1 (Figure 3.1b) because the effective rate constant, *k/R(A)*, was identical during the two tests. The peaks of A, B, and T were again separated from each other chromatographically; however, in this case, the B peak was located closer to the well than the A peak. The chromatographic separation of A and B peaks during this test caused the FMB concentrations of A and B to deviate from their ideal transport-adjusted concentrations (Figure 3.3b). However, since the B peak was located closer to the well than the A peak, FMB-adjusted concentrations of A were lower than ideal concentrations of A and FMB-adjusted concentrations of B were higher than ideal concentrations of B. As a result, the *k* derived from FMB-adjusted concentrations of A (0.082 day^{-1}) was high relative to the input *k* (0.069 day^{-1}). The error quotient, which in this case was defined as the derived *k* to the input *k* so that it wouldn't have to be expressed as a fraction, was 1.2. The error quotient obtained in test 3 was smaller than that obtained in test 2 because the degree to which A and B were chromatographically separated was less in test 3 than in test 2. Results from this simulated test indicate that in situ transformation rates estimated from field test data may be high by a factor of less than 1.5 when field conditions are similar to those used in this simulation.

Test Set II

The effects of test duration, groundwater velocity, input *k*, and dispersivity on the accuracy of derived rates were determined for cases in which *R(A)* and *R(B)* were 5 and 1.25, respectively, and 5 and 20, respectively. From this point forward, the error quotient is defined as the ratio of the input *k* to the derived *k* for all cases in which *R(A)* is greater than *R(B)* and the ratio of the derived *k* to the input *k* for all cases in which *R(A)* is less than *R(B)*. Error quotients increased as test duration (Figure 3.4a) or groundwater velocity (Figure 3.4b) increased since increases in these parameters led to increases in the chromatographic separation of A and B. Error quotients decreased as the input *k* increased (Figure 3.4c) because faster rates allowed more of the conversion of A to B to occur before chromatographic separation became significant. Error quotients also decreased with

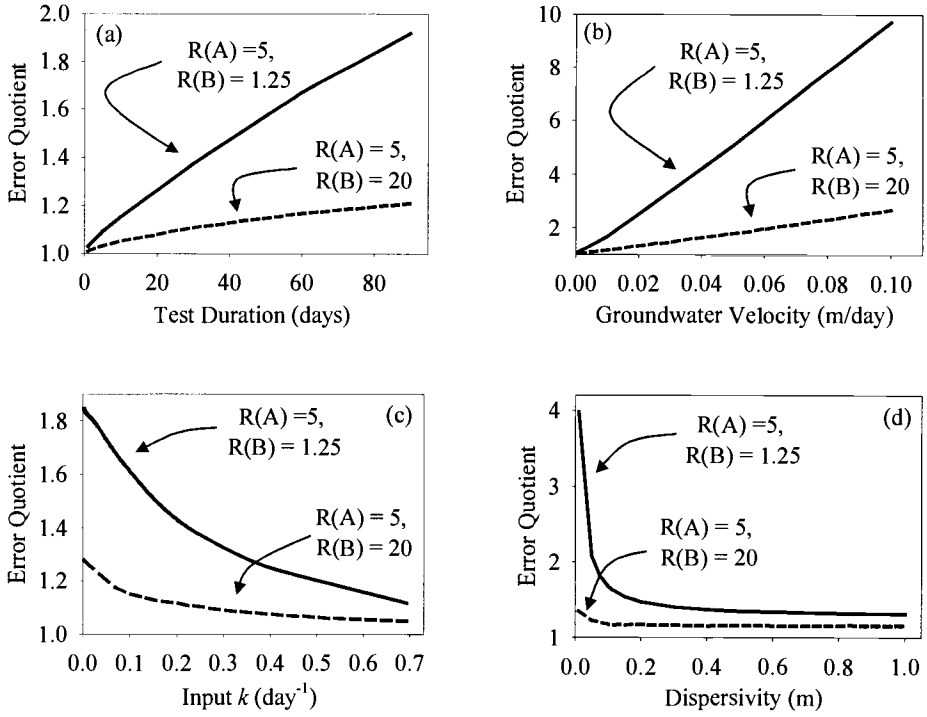

Figure 3.4: Test Set II. Error quotients as a function of (a) test duration, (b) groundwater velocity, (c) input *k*, (d) dispersivity.

increasing dispersivity (Figure 3.4d) because dispersivity affected the peak widths of A and B. As A and B peaks became more narrow (i.e. as dispersivity decreased), the overlap of the two peaks decreased and the ratio of the total concentrations of A to B at the well increasingly deviated from what it would have been if A and B were transported identically.

Test Set III

The effect of retardation factor values on the accuracy of derived rates was assessed from test set III simulations. For cases in which *R(A)* was greater than *R(B)*, error quotients became increasingly larger as the ratio of *R(A)* to *R(B)* increased for any given *R(A)* value (Figure 3.5a). Again, chromatographic separation was responsible for error. For example, the chromatographic separation of A and B was significantly greater when *R(A)* and *R(B)* were 50 and 1, respectively, (Figure 3.5b) than when they were 5 and 1.25, respectively (Figure 3.2a). Error quotients increased as *R(A)* decreased for a given *R(A)* to *R(B)* ratio (Figure 3.5a) because differences in transport velocities between A and B increased as *R(A)* decreased for a given *R(A)* to *R(B)* ratio.

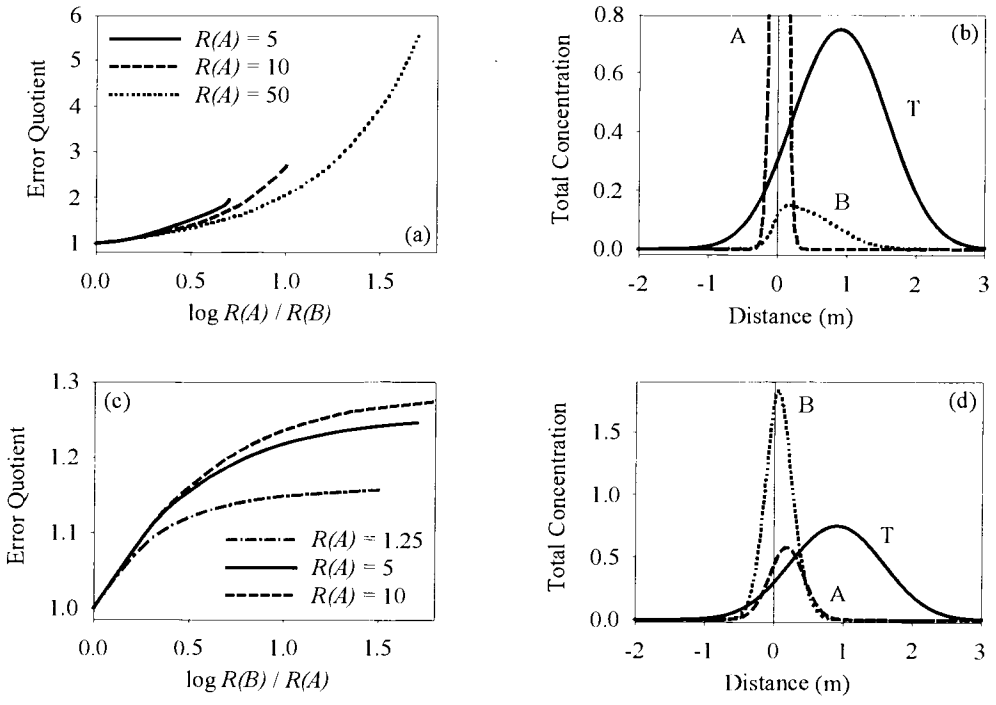

Figure 3.5: Test Set III. (a) Error quotients as they relate to retardation factor when $R(A) > R(B)$. (b) Total concentrations of A, B, and T in the simulated aquifer at 90 days when $R(A) = 50$ and $R(B) = 1$. (c) Error quotients as they relate to retardation factor when $R(A) < R(B)$. (d) Total concentrations of A, B, and T in the simulated aquifer at 90 days when $R(A) = 5$ and $R(B) = 250$.

For cases in which $R(A)$ was less than $R(B)$, error quotients began to level off after an initial increase as the ratio of $R(B)$ to $R(A)$ increased for any given $R(A)$ value (Figure 3.5c). Maximum error quotients were small relative to those observed when $R(A)$ was greater than $R(B)$ because the chromatographic separation of A and B was minimal during all cases in which $R(A)$ was less than $R(B)$. For example, A and B did not undergo significant chromatographic separation even when $R(A)$ and $R(B)$ were 5 and 250, respectively, (Figure 3.5d). Error quotients increased as $R(A)$ increased for a given $R(A)$ to $R(B)$ ratio (Figure 3.5c). This trend cannot be attributed to differences in transport velocities between A and B since they increased as $R(A)$ decreased for a given $R(A)$ to $R(B)$ ratio. Thus, R must have played a different, more important role in affecting errors when $R(A)$ was less than $R(B)$. One explanation is that errors increase with increasing $R(A)$ because R influences the effective rate constant (k/R) and the effective dispersivity (dispersivity/R), which both influence error (test set II).

FIELD APPLICATION

Concentrations of TCFE and its transformation products, DCFE (*cis*- and *trans*-), CFE, and FE were measured in extraction samples collected during a field push-pull test. The test was conducted by injecting a test solution containing TCFE into TCE-contaminated groundwater. Total (aqueous plus sorbed) concentrations for each solute except FE, which was not detected, were calculated from their measured concentrations using equations analogous to eqs 6 and 7. The values used in these calculations for K_{om} were given in the methods section and the values used for f_{om} (0.001), n (0.2), and ρ_g (2.9 kg/L) were determined from aquifer material collected at the field site. FMB-adjusted concentrations were calculated for each solute using eq 4 (Figure 3.6a). The best fit slope of ln ($[TCFE]_{FMB}/[TCFE]_{FMB,o}$) versus time (Figure 3.6b), where $[TCFE]_{FMB,o}$ is the injected FMB-adjusted concentration of TCFE, was 0.079 day^{-1} between 0 and 30 days. The field-derived k was calculated by multiplying this slope by the retardation factor of TCFE, *R(TCFE)*, which was 2.05 (see methods section). Hence, the field-derived k for the transformation of TCFE to DCFE was 0.16 day^{-1}.

Error estimates from the sensitivity analysis performed in test sets I-III were then used to qualitatively assess the accuracy of the field-derived k. First, it is likely that the field-derived k is underestimated relative to the actual in situ k since the retardation factor of TCFE is greater than that of its products. In addition, the magnitude of the error is likely to be less than a factor of two since data from the first 30 days of the test were used to estimate k (Figure

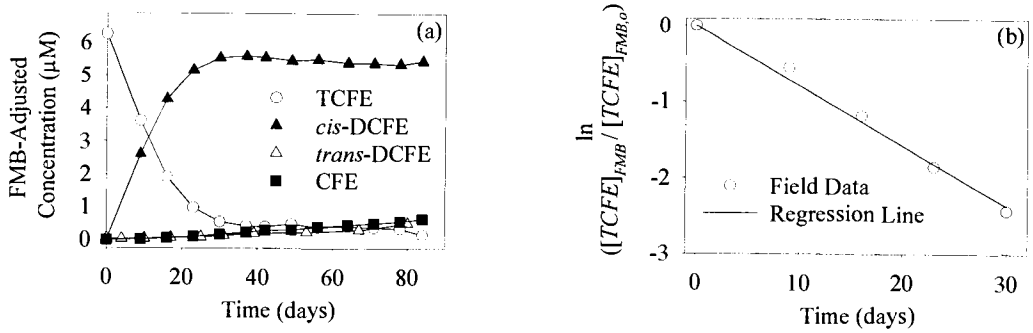

Figure 3.6: (a) FMB-adjusted concentrations of TCFE and its products, indicating that reductive dechlorination of TCFE occurred during this field push-pull test conducted in TCE-contaminated groundwater and (b) the plot from which the derived first-order rate constant was determined.

3.4a), the groundwater velocity was ~0.01 m/day (Figure 3.4b); the estimated input (or in situ) k was ~0.16 day^{-1} (Figure 3.4c), and the dispersivity in the aquifer was ~0.1 m (Figure 3.4d). The field-derived k is also expected to be in error by a factor of less than two than since the log *R(TCFE)/R(DCFE)* value was 0.17 (Figure 3.5a).

The accuracy of the field-derived k was assessed quantitatively by simulating push-pull tests with a test solution containing TCFE. Simulations were conducted with a range of input k values (0.07-0.26 day^{-1}) that bracketed the field-derived k. Derived k values were determined for each simulation using the FMB technique. The empirical relationship between the model input and derived k values for the transformation of TCFE to DCFE was

$$input\ k = 1.1 * derived\ k + 0.0080, \qquad (10)$$

which indicates that the field derived k was underestimated relative to the actual in situ k by a factor of 1.1, or 10%. If this error quotient is applied to the field-derived k, the estimated in situ k becomes 0.18 day^{-1}.

The potential effects of non-equilibrium sorption, aquifer property heterogeneities, and reaction rate heterogeneity on derived rates were not evaluated in this study. These issues will be investigated in future research projects. Nonetheless, to the best of our knowledge, the FMB technique makes it possible to estimate in situ transformation rates of sorbing solutes from push-pull test data for the first time. Moreover, errors in rates derived using the FMB technique can be quantified by coupling the technique to computer modeling.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION: EXAMPLE INPUT FILE FOR USE WITH STOMP

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# ----------------------------------------
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Forced Mass Balance Test 080802j,
Kimberly Hageman,
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08 August 2002,
12:30 pm PDT,
6,
Simulate T(racer), A, and B transport with 1st-order-aq,
P diff = 9.8 Pa, HC = 40 m/day, pw velocity = 0.01 m/day,
Dispersivity = 0.1 m, 0.004 m,
R(A) = 5, R(B) = 5,
Storativity = 0,
Half-life for A = 10 days,

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# ----------------------------------------
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125,min,90,day,0.05,min,0.05,day,1.25,8,1.e-06,
10000,
0,

# ----------------------------------------
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# ----------------------------------------
Cartesian,
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0,m,400@0.05,m,
0,m,1,m,
0,m,1,m,

# ----------------------------------------
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# ----------------------------------------
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# ----------------------------------------
~Aqueous Relative Permeability Card
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RichSoil,Mualem,,

# ----------------------------------------
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A,Conventional,1.0E-9,m^2/s,Continuous,10,day,
B,Conventional,1.0E-9,m^2/s,Continuous,1.0E10,yr,
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A,B,1,

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T,0,liter/kg,
A,0.3448,liter/kg,
B,0.3448,liter/kg,

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Solute Aqueous Conc.,A,0,1/liter,,,,,,,1,400,1,1,1,1,
Solute Aqueous Conc.,B,0,1/liter,,,,,,,1,400,1,1,1,1,

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East,Dirichlet Aqueous,Outflow,Outflow,Outflow,
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Solute Aqueous Conc.,B,1/liter,
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60,day,
90,day,
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Solute Aqueous Conc.,A,1/liter,
Solute Aqueous Conc.,B,1/liter,
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CHAPTER 4. QUANTIFYING THE EFFECTS OF CHEMICAL AMENDMENTS ON IN SITU REDUCTIVE DECHLORINATION RATES

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ABSTRACT

In situ methods are needed to evaluate the effectiveness of chemical amendments at enhancing reductive dechlorination rates in groundwater that is contaminated with the priority pollutant, trichloroethene (TCE). In this communication, a method that utilizes single-well, "push-pull" tests to quantify the effects of chemical amendments on in situ reductive dechlorination rates is presented and demonstrated. Five push-pull tests were conducted in each of five monitoring wells located in a TCE-contaminated aquifer at the site of a former pesticide-manufacturing site. Rates for the reductive dechlorination of the fluorinated TCE-surrogate, trichlorofluoroethene (TCFE), were measured before (test 1) and after (test 5) three successive additions (tests 2-4) of fumarate, which was selected to stimulate the growth and activity of indigenous microorganisms with the metabolic capability to reduce TCFE and TCE. In three wells, first-order rate constants for the reductive dechlorination of TCFE increased by 8.9 to 120 times following fumarate additions. In two wells, reductive dechlorination of TCFE was observed after fumarate additions but not before. The transformation behavior of fumarate was also monitored following each fumarate addition. Correlations between the reductive dechlorination of TCFE and the reduction of fumarate to succinate were observed, indicated that these reactions were supported by similar biogeochemical conditions at this site.

INTRODUCTION

Significant research efforts have been devoted to the development of in situ bioremediation as an approach for remediating groundwater contaminated with the priority pollutant, trichloroethene (TCE) and other chlorinated aliphatic hydrocarbons. In anaerobic environments, this approach depends on the metabolic capability of indigenous subsurface microorganisms to catalyze the reductive dechlorination of TCE to the dichloroethene (DCE) isomers, chloroethene (CE), and ethene (*1-3*) (Figure 4.1a). Engineered approaches are needed where natural attenuation does not result in the complete conversion of TCE to ethene or where rates are too slow to meet risk management goals.

A common approach for enhancing in situ reductive dechlorination is to stimulate the growth of indigenous dechlorinating microorganisms with the addition of chemical amendments. A wide variety of chemicals and chemical mixtures have been evaluated for their suitability as amendments for enhancing reductive dechlorination. Lee et al. reviewed results from laboratory tests that were designed to assess the effectiveness of potential amendments such as complex organic mixtures (molasses, wastewater, cheese whey permeate, corn steep liquor, manure tea), metabolic intermediates (benzoate, lactate, propionate, acetate, butyrate), alcohols (methanol, ethanol), molecular hydrogen, sulfate, nitrate, vitamins, and micronutrients (*4, 5*). While many of these amendments were effective, disadvantages were associated with each and none were universally effective at stimulating reductive dechlorination in groundwater from all sites. To the best of our knowledge, fumarate (*trans-*

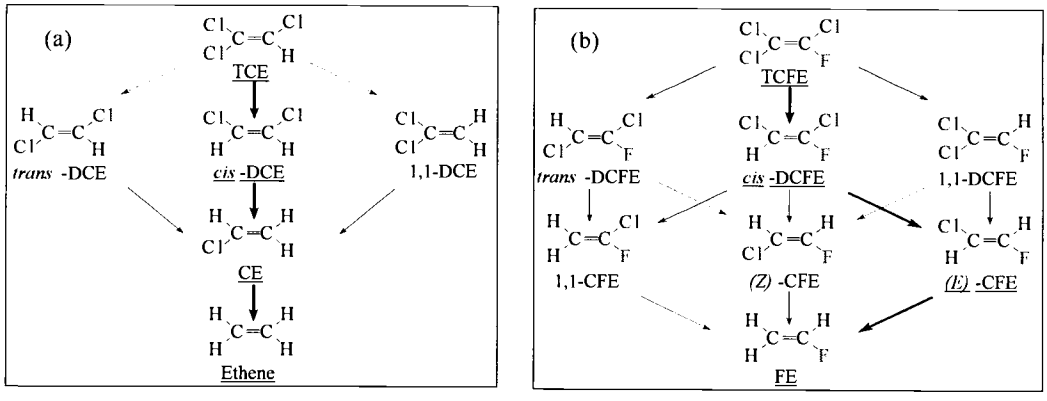

Figure 4.1. Analogous reductive dechlorination pathways for (a) TCE and (b) its fluorinated surrogate, TCFE. Predominant isomers and pathways are indicated by underlines and heavy arrows.

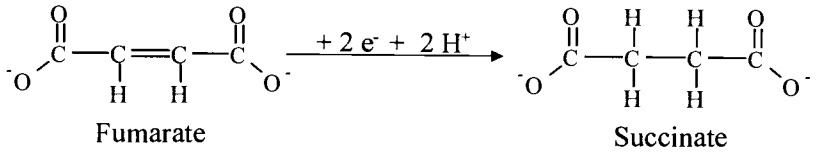

Figure 4.2. Reduction of fumarate to succinate.

1,2-ethenedicarboxylate) has not previously been tested as a chemical amendment for enhancing reductive dechlorination rates. There is evidence that a number of dechlorinating microorganisms use fumarate as an alternative electron acceptor (*6-13*) and that certain dechlorinating organisms grow faster on fumarate than on chlorinated ethenes (*11*). Some dechlorinating microorganisms are also known to use fumarate (*14*) or succinate (1,2-ethanedicarboxylate) (*9*), which is produced from the reduction of fumarate (Figure 2), as electron donors during reductive dechlorination. Hence, one objective of this work was to evaluate the effectiveness of fumarate at enhancing in situ reductive dechlorination rates.

The effectiveness of a chemical amendment is generally evaluated by comparing reductive dechlorination rates measured with and without the chemical amendment in laboratory experiments with pure or mixed cultures of microorganisms. Experiments conducted in actual aquifers are not as common because field experiments, especially those involving well-to-well tests, are perceived as complicated, expensive, and/or time-consuming in comparison to laboratory experiments. In addition, in situ transformation rates are difficult to measure because solute concentrations in groundwater are affected by both transformation and transport (advection, dispersion, and sorption) processes. Nevertheless, the need for field methods that can be used to evaluate the effectiveness of chemical amendments becomes increasingly apparent as concerns about discrepancies between laboratory and field results mount (*15-17*). Field pilot tests designed to determine in situ reductive dechlorination rates in the presence of chemical amendments (e.g. acetate, nitrate, and sulfate) were reviewed by Lee et al. (*5*). In all but one of the thirteen reported pilot tests, in situ reductive dechlorination rates were determined using multi-well tests that involved the recirculation of amended groundwater between injection and extraction wells.

Hageman et al. recently described an alternative method for determining in-situ reductive dechlorination rates (*18*) that utilizes single-well, "push-pull" test technology (*19*). Push-pull tests were conducted in a TCE-contaminated aquifer at the site of former pesticide-

manufacturing plant in the San Francisco Bay area. Each push-pull test was conducted by injecting ("pushing") an aqueous test solution into the saturated zone via a monitoring well. Test solutions contained bromide, which served as a conservative tracer; trichlorofluoroethene (TCFE), which served as a surrogate for TCE; and in some cases, formate, which is a common chemical amendment used as an electron donor. TCFE was selected as a surrogate for TCE based on evidence that it undergoes reductive dechlorination by a pathway analogous to that of TCE while retaining the fluorine label (Figure 4.1b) (*20*). TCE itself was not injected because mixing of the injected test solution with native groundwater would have rendered it impossible to distinguish injected and background TCE. Following the injection phase, samples of the test solution/groundwater mixture were periodically extracted ("pulled") from the single well and analyzed for TCFE, transformation product, and tracer concentrations. TCFE reductive dechlorination rates were determined from measured concentrations that had been adjusted with a data processing technique designed to remove the effects of transport processes. Hageman et al. discussed reductive dechlorination rates obtained in three wells at the site (*18*). In one of those wells, the effect of formate on in situ reductive dechlorination rates was quantified by comparing the rate obtained during a test conducted by co-injecting TCFE and formate to the rate obtained in a later test conducted by injecting TCFE without formate.

There are a number of advantages associated with using push-pull tests instead of multi-well tests to evaluate chemical amendments. For instance, push-pull tests take less time to conduct than multi-well tests since injected solutes do not have to be transported between wells. Because push-pull tests are time-efficient, push-pull tests can be repeated in a single well with and without a co-injected chemical amendment or before and after a suite of chemical amendment additions to the well. At sites where a number of monitoring wells exist, push-pull tests can be conducted simultaneously in different wells to determine the effects of different amendments on transformation rates or to assess spatial variability in transformation rates. Push-pull tests are cost-effective relative to multi-well tests because fewer groundwater wells, which are expensive to construct, are needed. Moreover, a revised form of the data processing technique designed to remove the effects of transport processes from measured concentrations of sorbing solutes was recently presented (*21*). The availability of this data processing technique makes it possible to obtain in situ transformation rates from push-pull test data for a larger selection of reactants.

The objective of this study was to quantify the effects of a specific chemical amendment, namely fumarate, on in situ reductive dechlorination rates of TCFE using push-pull tests. To this end, TCFE reductive dechlorination rates were measured before and after three consecutive additions of fumarate in five wells. Additionally, the transformation behavior of fumarate was monitored after each fumarate additions so that potential correlations between reductive dechlorination and fumarate transformation behavior could be assessed.

EXPERIMENTAL SECTION

Chemicals

Trichlorofluoroethene (TCFE) (97% pure, containing 0.1% *cis*-DCFE and 0.3% *trans*-DCFE), *cis/trans*-1,2-dichloroethene (DCFE) (98%), and *E/Z*-1-chloro-2-fluoroethene (97%) were obtained from SynQuest Laboratories, Inc. (Alachua, FL). Fluoroethene (FE) (98%) was obtained from Lancaster Synthesis (Pelham, NH). Sodium fumarate (98%) and sodium succinate (99%) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Potassium bromide (99.7%) and sodium formate (99.6%) were obtained from Fisher Scientific (Fair Lawn, NJ). For use as an internal standard, 1-chloropropane was obtained from Matheson Company (Cincinnati, OH).

Site Description

Push-pull tests were conducted at the site of a former pesticide-manufacturing plant in the San Francisco Bay area where reductive dechlorination has been monitored in recent years (*18, 22, 23*). Tests were conducted in two distinct aquifer zones. The A-zone is an unconfined shallow layer composed mainly of placed fill over Bay Mud. The water table lies within 3 meters of the ground surface. The groundwater velocity ranges from 1.5 to 6 meters per year. The C-zone underlies the Bay Mud and is characterized by alluvial fan deposits located approximately 6 to 23 meters below the ground surface. Groundwater velocities range from 6 to 31 meters per year.

Push-Pull Tests

A series of five push-pull tests were conducted in each of two A-zone wells (10A and 9A) and in each of three C-zone wells (15C, 16C, and 21C) between December 1999 and February 2001. The objective of test 1 was to measure initial rates for TCFE reductive dechlorination. Therefore, injected test solutions contained TCFE and bromide (Table 4.1), which served as a conservative tracer. Fumarate was included in the test solution injected into

well 9A but not 10A so that reductive dechlorination rates could be compared with and without co-injected fumarate. Formate was included as an electron donor in test solutions injected into wells 15C and 16C because results from a previously conducted screening study (*23*) indicated that reductive dechlorination was limited by electron donor availability in the C-zone. The objectives of tests 2-4 were to amend the TCE-contaminated groundwater with fumarate and then to monitor the fumarate transformation behavior following each addition. Thus, test solutions for tests 2-4 contained fumarate and bromide (Table 4.1). The objective of test 5 was to measure reductive dechlorination rates after the fumarate additions so that rate changes due to fumarate could be quantified. Therefore, TCFE, bromide, fumarate, and formate were injected in test 5 at concentrations similar to those used in test 1 (Table 4.1).

The experimental design was identical to that described previously (*18*). Briefly, test solutions were prepared on-site by adding bromide and fumarate or formate (Table 4.1) to argon-sparged tap water. In tests 1 and 5, a concentrated aqueous solution of TCFE was metered into the injection line. The TCFE solution was stored and pumped from a collapsible metallized-film gas-sampling bag to prevent volatilization loss during injection. Following the injection, samples of the test solution/groundwater mixture were collected approximately once per week for up to 85 days during tests 1 and 5 and on a varying schedule, with a maximum of 4 samples per day, for up to 20 days during tests 2-4.

Analytical Methods

Chromatographic separation and detection of TCFE, DCFE, CFE, and FE were achieved using a gas chromatography/mass spectrometry method that was previously described (*18*). The one difference was that purge and trap was used as the analyte introduction method with test 5 samples. The purge and trap system was composed of a Tekmar-Dohrmann (Cincinnati, OH) 3100 sample concentrator and an AQUA Tek 70 liquid analyzer. Chromatographic separation and detection of organic acids in test series 1-4 samples were performed on a Waters Alliance (Milford, MA) high performance liquid chromatograph equipped with a photodiode array detector. Separations were achieved on a Phenomenex (Torrance, CA) Luna C18 column. Test series 5 samples were analyzed on a Dionex (Sunnyvale, CA) DX-320 ion chromatograph equipped with a conductivity detector and AS11 column. Bromide concentrations were measured with either a Dionex DX-120 or

Table 4.1. Test Solution Compositions

Well	Test 1 (12/99)	Test 2 (9/00)	Test 3 (10/00)	Test 4 (12/00)	Test 5 (2/01)
10A	13 μM TCFE 1.3 mM bromide	0.81 mM fumarate 1.3 mM bromide	0.12 mM fumarate 1.3 mM bromide	0.75 mM fumarate 1.4 mM bromide	9.4 μM TCFE 1.1 mM bromide
9A	5.3 μM TCFE 1.3 mM bromide 0.75 mM fumarate	0.87 mM fumarate 1.3 mM bromide	0.13 mM fumarate 1.3 mM bromide	0.74 mM fumarate 0.65 mM bromide	4.3 μM TCFE 1.3 mM bromide 0.70 mM fumarate
15C	16 μM TCFE 1.3 mM bromide 8.3 mM formate	0.79 mM fumarate 1.2 mM bromide	0.13 mM fumarate 1.3 mM bromide	0.74 mM fumarate 1.4 mM bromide	15 μM TCFE 1.3 mM bromide 6.2 mM formate
16C	14 μM TCFE 1.3 mM bromide 8 mM formate	0.81 mM fumarate 1.2 mM bromide	0.14 mM fumarate 1.4 mM bromide	0.73 mM fumarate 1.3 mM bromide	11 μM TCFE 1.3 mM bromide 6.3 mM formate
21C	33 μM TCFE 1.4 mM bromide	0.81 mM fumarate 1.3 mM bromide	0.12 mM fumarate 1.3 mM bromide	0.70 mM fumarate 0.98 mM bromide	29 μM TCFE 1.3 mM bromide

Dionex DX-320 ion chromatograph equipped with a conductivity detector and Dionex AS14 or AS11 column.

Data Analysis

In situ rates for the reductive dechlorination of TCFE were determined by removing the effects of transport processes from measured aqueous concentrations of TCFE using a data processing technique called "forced mass balance" (FMB) (*21*). TCFE transformation products were also treated with the FMB technique so that the distribution of products formed in situ could be readily compared between tests. The FMB technique was selected over other available data processing techniques (*24, 25*) because it was designed for use with sorbing solutes. Short-term transport tests conducted at the selected site (*18*) as well as calculated retardation factor values for TCFE indicated that sorption could affect TCFE transport in both A- and C-zones. The retardation factor (R) values calculated for TCFE in the A- and C-zones were 11.5 and 2.05, respectively. These values were calculated (*26*) using a K_{om} value for TCFE of 90.5 L/Kg, bulk density of 2.32 Kg/L, aquifer porosity of 0.2, and fraction organic matter values of 0.01 (A-zone) and 0.001 (C-zone). The K_{om} value was estimated using the Estimations Program Interface Suite (*27*) while the other values were selected based on measurements made on aquifer solids collected at the site.

Briefly, the FMB technique is conducted by first calculating the mass of the solute in the aqueous sample obtained during the extraction-phase of the push-pull test. The second step is to calculate the mass of solute in the sorbed phase associated with the extraction sample. The third step is to calculate the total (aqueous plus sorbed) concentration of the solute by dividing the sum of the aqueous-phase and sorbed-phase masses by the sum of the volumes of the aqueous extraction sample and the associated sorbed-phase. Finally, FMB-adjusted concentrations are obtained by dividing each total concentration by the adjustment factor, Σ/Σ_o, where Σ and Σ_o are the sums of the total concentrations of TCFE and its transformation products in the extraction sample and in the injected test solution, respectively. TCFE transformation rates are then determined from its FMB-adjusted concentrations. The validity of the FMB technique was evaluated by quantifying errors in rates derived by applying FMB to push-pull test data generated by a numerical model (*21*). Additionally, numerical modeling was used to quantify the error in the transformation rate obtained from push-pull test data obtained during test 5 conducted in well 15C from this study. Since the error analysis indicated that the in situ rate for the reductive dechlorination of TCFE was

underestimated relative to the true in situ rate by 10%, all rates reported herein are expected to be underestimated by a similar magnitude.

The effects of transport processes were removed from measured aqueous concentrations of fumarate, succinate, and formate using a data processing technique for use with nonsorbing solutes hereafter referred to as "tracer-normalization" (*24, 25*). Sorption was assumed to have a minimal effect on fumarate, succinate, and formate concentrations since these solutes are negatively charged and highly water-soluble. Tracer-normalized concentrations for each solute were obtained by dividing their measured aqueous concentrations by the adjustment factor, $[Br]/[Br]_o$, where [Br] and $[Br]_o$ are the measured bromide concentrations in an extraction sample and in the injected test solution, respectively.

RESULTS AND DISCUSSION

Reductive Dechlorination Rates and Product Distribution Ratios

Reductive dechlorination of TCFE occurred following its first injection into well 10A (test 1, Table 4.1) as indicated by decreasing aqueous TCFE concentrations and increasing aqueous cis-DCFE concentrations (Figure 4.3a). *Trans*-DCFE and CFE were also detected at relatively low concentrations (< 0.05 μM). The FMB technique (see methods section) was used to obtain adjusted concentrations of TCFE and its transformation products that reflect the effects of transformation and not transport (Figure 4.3b). The first-order rate constant for the transformation of TCFE to DCFE was determined by plotting ln $([TCFE]_{FMB}/[TCFE]_{FMB,o})$ values versus time (Figure 4.3c), where $[TCFE]_{FMB}$ and $[TCFE]_{FMB,o}$ are the FMB-adjusted concentrations of TCFE in an extraction sample and in the injected test solution, respectively. If it is assumed that reductive dechlorination occurs in the aqueous phase only (and not in the sorbed phase), then the best-fit slope of this plot represents the first-order rate constant (k) divided by the retardation factor (R) of TCFE. Hence, the slope (0.0010 day^{-1}) was multiplied by the R of TCFE (11.5, see methods section) to obtain an in situ reductive dechlorination rate of 0.012 day^{-1} (Table 4.2). FMB-adjusted concentrations were also plotted in a stacked area graph (Figure 4.4a) so that the distribution ratios of TCFE and its products could be more easily visualized.

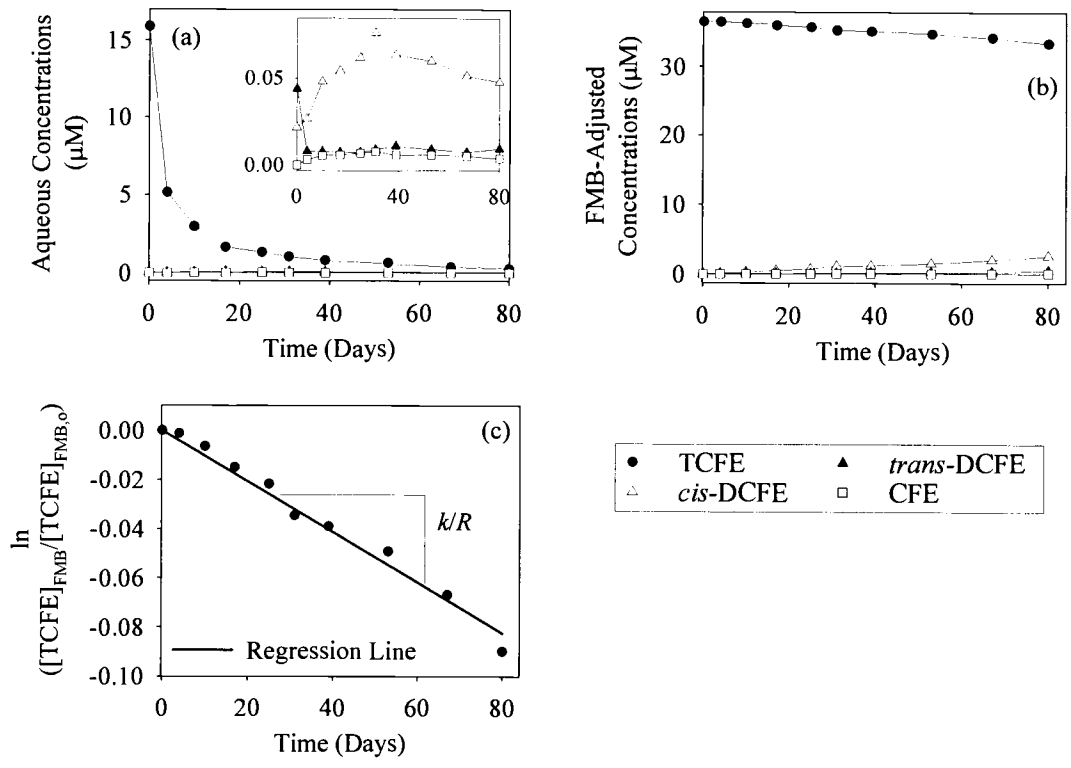

Figure 4.3. Test 1 in Well 10A. (a) Aqueous measured concentrations illustrating concentration changes due to transformation and transport processes, (b) forced mass balance (FMB)-adjusted concentrations (see data analysis section) illustrating concentration changes due to transformation only and (c) the plot used to determine the first-order rate constant (k).

After three successive additions of fumarate (tests 2-4), which were made 1-2 months apart from each other, TCFE was injected into well 10A for a second time (test 5). Reductive dechlorination of TCFE to *cis*-DCFE, *trans*-DCFE, CFE, and FE occurred (Figure 4.4b) with TCFE being transformed at a maximum rate of 1.4 day^{-1} between days 30 and 71 (Table 4.2). Thus, the maximum transformation rate was ~120 times greater after fumarate additions than before. Moreover, the extent of dechlorination increased, as indicated by the detection of the less chlorinated products. The formation of FE had important implications for bioremediation at this site because it is the fluorinated analog of ethene (*18, 28*) (Figure 4.1) and ethene is the desired end product for reductive dechlorination due to its lack of toxicity.

Table 4.2. TCFE Transformation Rates

Well	Maximum First-Order Rate Constants for TCFE Transformation (day^{-1})[a]	
	Test 1 (12/99):	**Test 2 (2/01):**
10A	0.012 (0-80 days)	1.4 (30-71 days)
9A	0.022 (0-84 days)	1.8 (7-23 days)
15C	0.018 (55-82 days)	0.16 (0-30 days)
16C	--	0.047 (49-84 days)
21C	--	0.14 (16-49 days)

[a]Transformation rates were calculated from FMB-adjusted concentrations (see data analysis section).

Following the first injection of TCFE into well 9A (test 1), TCFE underwent reductive dechlorination primarily to *cis*-DCFE although *trans*-DCFE was also formed (Figure 4.4c). The rate of TCFE transformation was 0.022 day^{-1} (Table 4.2). Note that fumarate was co-injected with TCFE during the initial test conducted in well 9A but not during the initial test conducted in well 10A. However, the co-injection of fumarate did not appear to affect initial reductive dechlorination rates since initial rates in wells 9A and 10A were similar (Table 4.2). After three additional injections of fumarate (tests 2-4) to well 9A, TCFE and fumarate were co-injected into well 9A for a second time (test 5). TCFE underwent reductive dechlorination primarily to *cis*-DCFE although *trans*-DCFE and CFE were also formed (Figure 4.4d). TCFE was transformed at a maximum rate of 1.8 day^{-1} between days 7 and 23 (Table 4.2). Thus, the maximum transformation rate was 82 times greater during test 5 than during test 1. While

TCFE transformation rates obtained during test 5 in wells 10A and 9A were similar, the extent of dechlorination observed in these wells was remarkably different. In well 9A, *cis*-DCFE was formed almost exclusively while in well 10A, the dechlorination of TCFE to FE

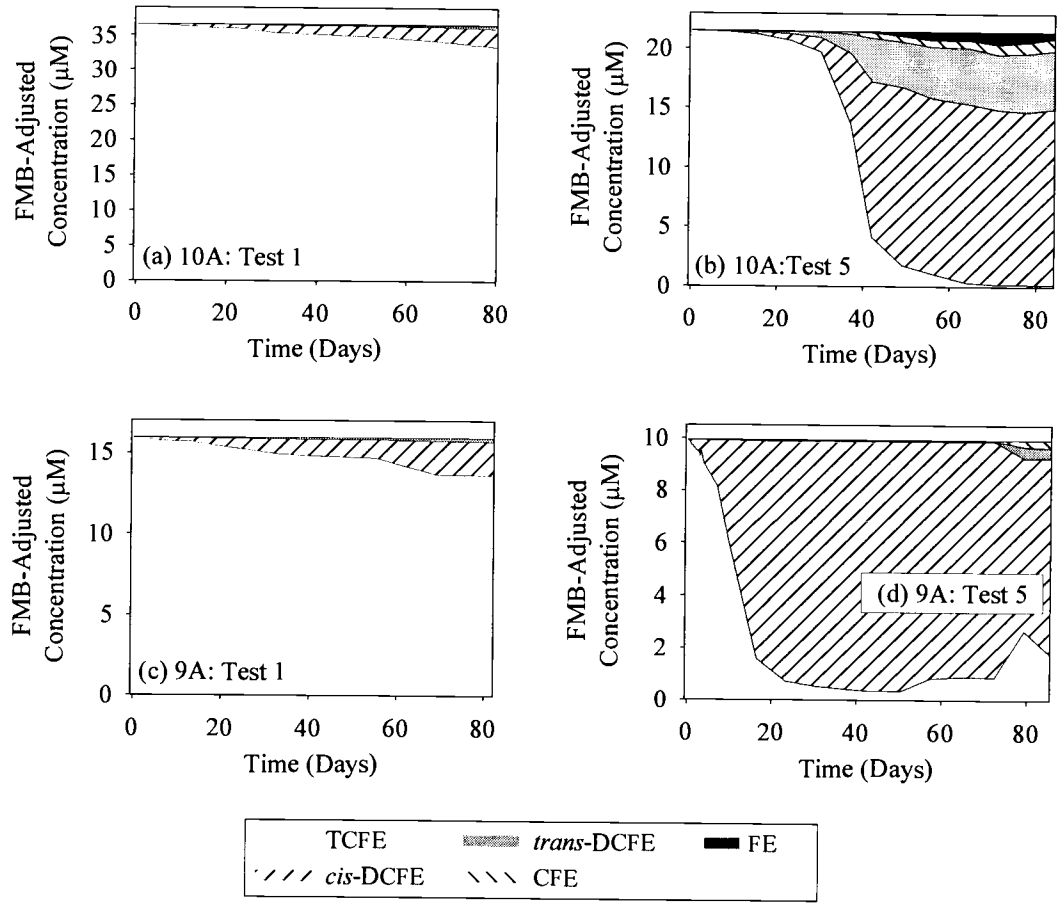

Figure 4.4. Stacked area plots of FMB-concentrations depicting reductive dechlorination of TCFE following the first (test 1) and second (test 5) injections of TCFE in A-zone wells.

was observed. Possible explanations are that (a) the co-injection of fumarate with TCFE inhibited further dechlorination or that (b) the microbial populations initially available for stimulation by fumarate were different in the two wells even though the wells were located within 25 m of each other and in the same horizontal subsurface zone.

Reductive dechlorination was not observed until 55 days after the first injection of TCFE into well 15C (test 1) (Figure 4.5a). At that point, TCFE underwent reductive dechlorination primarily to *cis*-DCFE although *trans*-DCFE was also formed. The maximum rate of TCFE transformation was 0.018 day^{-1} (Table 4.2). To increase the likelihood that reductive dechlorination would occur, formate was co-injected as an electron donor with TCFE during this test. Co-injected formate was completely degraded by day 27 (data not

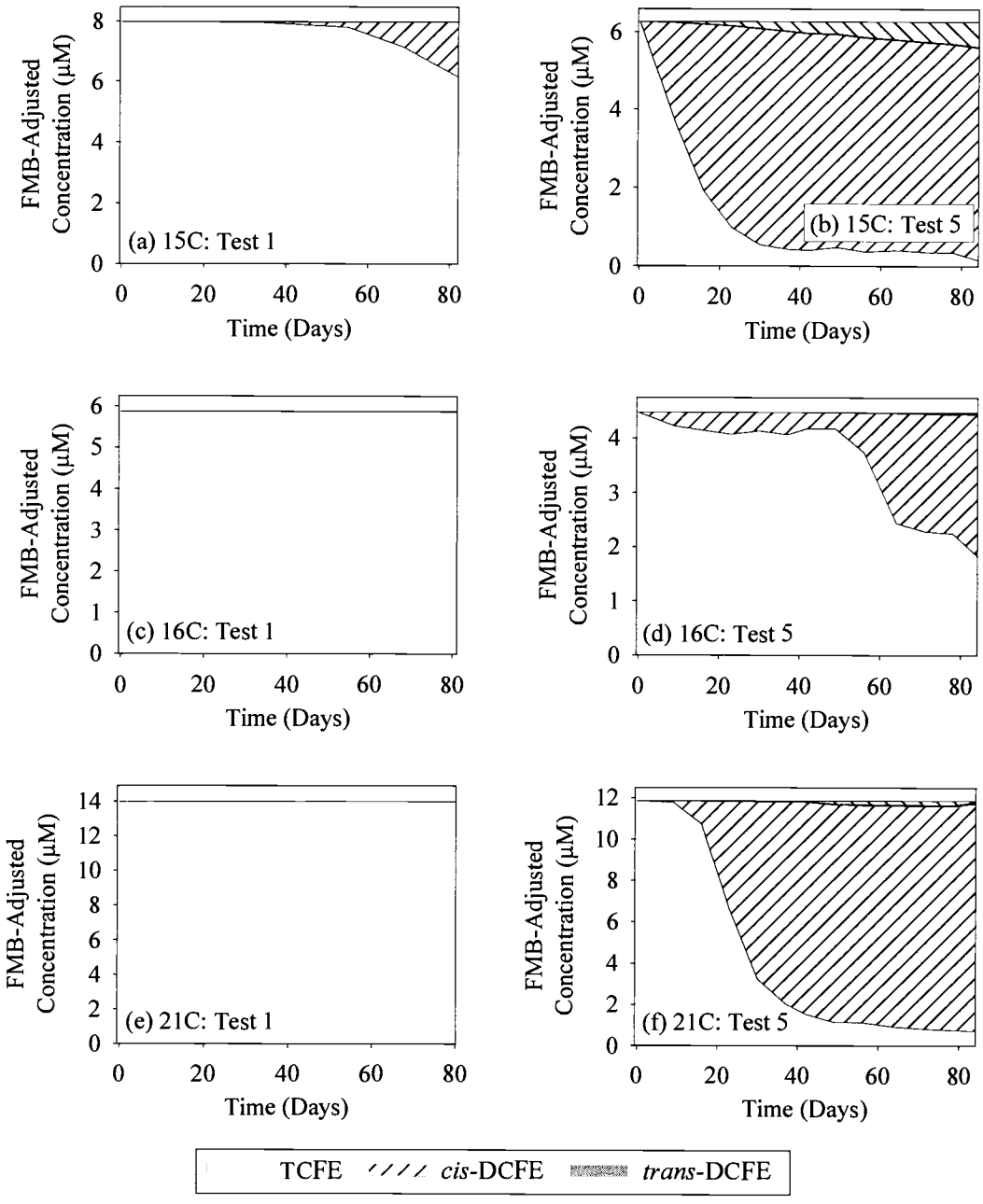

Figure 4.5. Stacked area plots of FMB-concentrations depicting reductive dechlorination of TCFE following the first (test 1) and second (test 5) injections of TCFE in C-zone wells.

shown), indicating that an active community of formate-utilizing organisms was present. However, acetate, which would have been formed during acetogenesis of injected formate, was not detected. After three successive injections of fumarate (tests 2-4), TCFE and formate

were co-injected into well 15C for a second time (test 5). Reductive dechlorination of TCFE to primarily *cis*- and *trans*-DCFE occurred (Figure 4.5b) although CFE was also formed. TCFE was transformed at a maximum rate of 0.16 day^{-1} between days 0 and 30 (Table 4.2). Thus, the maximum transformation rate was 8.9 times greater after fumarate additions than before. However, it may be of greater significance that the transformation reaction began immediately during test 5 instead of being delayed until late in the test as it was during test 1. Co-injected formate was not detected in any samples, indicating that the formate-utilization rate also increased between tests. This observation is consistent with the expectation that electron-donor utilization and reductive dechlorination rates should increase together. Again, acetate was not detected.

In contrast to what was observed in all previously-described wells, reductive dechlorination did not occur after the first injection of TCFE into well 16C (test 1) (Figure 4.5c). Formate, which was again co-injected as an electron donor with TCFE, underwent degradation slowly such that it was still detected in samples collected at the end of the test (data not shown). This observation is consistent with the expectation that a slow formate-utilization rate would accompany a slow or non-detectable TCFE reductive dechlorination rate. Acetate formation via the potential acetogenesis pathway was not detected. After three successive additions of fumarate (tests 2-4), TCFE and formate were co-injected into well 16C for a second time (test 5). Reductive dechlorination of TCFE primarily to *cis*-DCFE occurred although *trans*-DCFE and CFE were also formed (Figure 4.5d). TCFE was transformed at a maximum rate of 0.047 day^{-1} between days 49 and 84 (t2). Co-injected formate was not detected, indicating that formate-utilization and TCFE reductive dechlorination rates increased together, as was observed in well 15C. Although relatively high concentrations of TCFE persisted until the end of the test, it is of particular significance that TCFE reductive dechlorination was stimulated where it had not initially been observed (test 1) (Figure 4.5c).

Well 21C is located upgradient from the contaminant plume in a location where background contamination does not exist. Reductive dechlorination of TCFE did not occur following the first injection of TCFE (test 1) (Figure 4.5e), which is consistent with the expectation that an active population of dechlorinating organisms would not exist in the absence of chlorinated compounds. After three successive additions of fumarate (tests 2-4), TCFE was injected for a second time (test 5). Reductive dechlorination of TCFE primarily to *cis*-DCFE occurred although *trans*-DCFE and CFE were also formed (Figure 4.5f). TCFE

was transformed at a maximum rate of 0.14 day^{-1} between days 16 and 49 (Table 4.2). The occurrence of TCFE reductive dechlorination in well 21C is of significance since it again demonstrates that fumarate additions can stimulate reduction dechlorination even where it was not initially observed.

Correlations between Fumarate and TCFE Transformation Behavior

Three types of fumarate transformation behavior were observed during successive fumarate additions (tests 2-4). The first type of behavior is classified as that in which injected fumarate was reduced to succinate at an increasing rate following each fumarate addition. This type of behavior was observed in wells 10A and 9A. In well 10A, for example, fumarate concentrations decreased more rapidly in test 4 than in test 2 (Figure 4.6a). Likewise, succinate formation was observed earlier in test 4 than in test 2. In both tests 2 and 4, succinate concentrations eventually decreased to undetectable levels, indicating that microbial populations utilized succinate. Based on results from laboratory experiments (*9*), it is likely

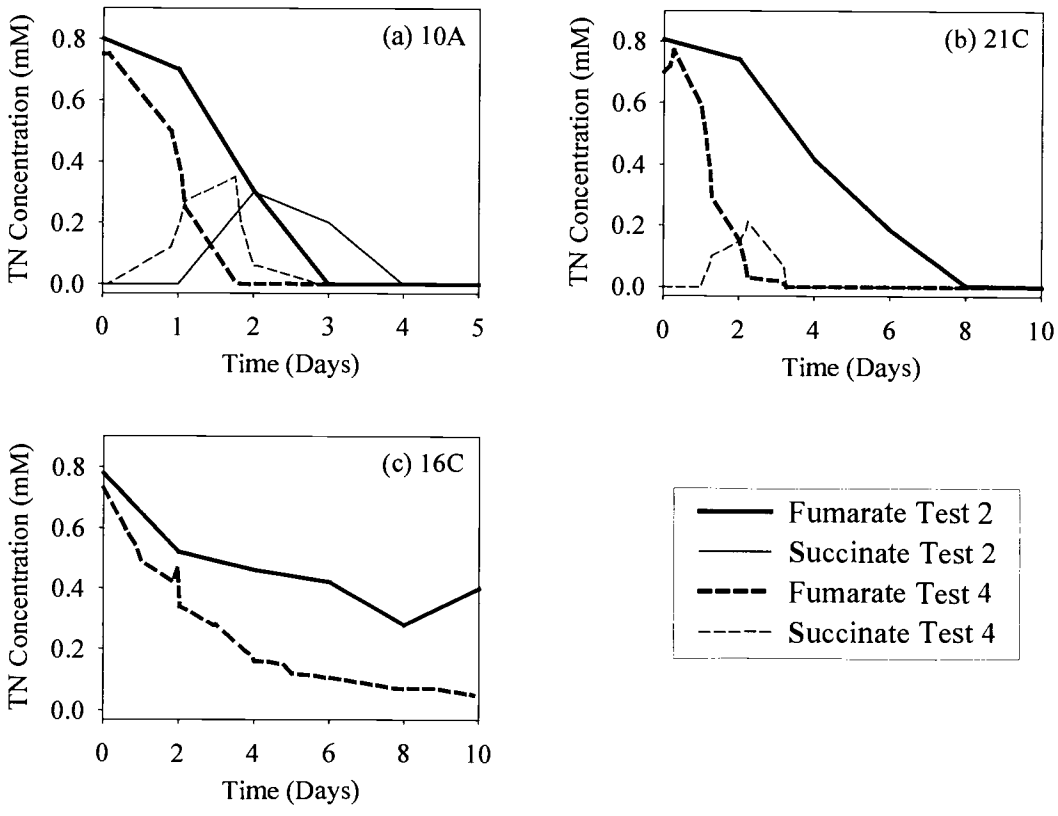

Figure 4.6. Tracer-normalized (TN) concentrations (see data analysis section) of fumarate and its reduction product, succinate.

that succinate was utilized as an electron donor. The transformation behavior exhibited by TCFE was similar to that exhibited by fumarate in that reductive dechlorination rates increased between tests 1 and 5. The similarities between TCFE and fumarate transformation behaviors indicate that reductive dechlorination and fumarate reduction may be supported by similar biogeochemical conditions in the A-zone.

The second type of fumarate transformation behavior is classified as that in which the reduction of fumarate to succinate was not observed after its first addition (test 2) but was observed after later additions (e.g. test 4). This type of behavior was observed in wells 15C and 21C. In well 21C, for example, fumarate concentrations decreased without the concomitant detection of succinate in test 1 (Figure 4.6b). However, decreases in fumarate concentrations were accompanied by succinate detection in test 4. While it is possible that succinate was not detected in test 2 because it was formed as quickly as it was reduced, the detection of succinate in test 4 indicates that microbial populations capable of reducing fumarate were stimulated by successive fumarate additions. The TCFE transformation behavior observed in wells 15C and 21C was consistent with that observed for fumarate since reductive dechlorination was either observed only after a significant lag time or not observed at all in these wells during test 1. Yet, reductive dechlorination was observed in these wells during test 5. This correlative behavior indicates that reductive dechlorination and fumarate reduction may also be supported by similar biogeochemical conditions in the C-zone.

The third type of fumarate transformation behavior is classified as that in which the reduction of fumarate to succinate was not observed during any of the successive fumarate additions (tests 2-4). This type of behavior was observed in well 16C only (Figure 4.6c). This behavior suggests that the biogeochemical conditions of well 16C were not conducive to the stimulation of fumarate-reducing microbial populations. Based on the correlations between reductive dechlorination and fumarate reduction observed in other wells, it is not surprising that fumarate additions had a smaller effect on reductive dechlorination rates in well 16C than in any other well.

Although TCFE and fumarate displayed parallel transformation behaviors in all five wells at this site, the mechanism that caused TCFE reductive dechlorination rates to increase is not known. A clue to understanding fumarate's role in enhancing reductive dechlorination may be found in the fact that fumarate as well as its reduction product, succinate, were short-lived in all wells except 16C (Figures 6a-c), where neither fumarate nor TCFE were

particularly susceptible to reduction. Because fumarate and succinate were short-lived, they were not present in the groundwater during the final TCFE injection (test 5) and therefore could not have been directly responsible for increased reductive dechlorination rates. Instead, the consecutive additions of fumarate appeared to have altered the biogeochemical conditions in a way that favored reductive dechlorination. Since the literature indicates that a number of dechlorinating microorganisms utilize fumarate as an alternative electron acceptor (*6-13*) and that at least one of them grows faster on fumarate than on chlorinated ethenes (*11*), it is possible that fumarate additions stimulated the growth of dechlorinating/fumarate-reducing microorganisms. This change in microbial community structure could have been responsible for the enhancement of reduction rates for both TCFE and fumarate. It is also possible that changes in microbial populations were induced by the presence of succinate (formed in situ), which can be used as an electron donor (*9*), or by fumarate acting as an electron donor instead of as an electron acceptor (*14*).

Another potential scenario is that by acting as an electron donor, fumarate reduced the concentrations of indigenous electron acceptors such as oxygen or sulfate that can compete with chlorinated ethenes. However, measured sulfate concentrations gave no indication that sulfate reduction was occurring during these tests (data not shown). In some cases, TCFE additions may also have contributed to increased TCFE reductive-dechlorination rates by stimulating the growth of dechlorinating microorganisms. For example, during test 5 in well 10A, where background chlorinated ethene concentrations were negligible, changes in TCFE reductive-dechlorination rates during the test followed a characteristic growth pattern with a lag period of about 30 days. In contrast, during test 5 in well 9A, TCFE concentrations decreased rapidly at the onset of the test, indicating that dechlorinating populations were already present in the well at the beginning of the test. Accelerating rates, which can indicate growth, were also observed during test 1 in well 15C and test 5 in well 21C. On-going research in our group with soil microcosms is designed to further characterize the relation between fumarate, TCFE, and TCE reductive dechlorination.

CONCLUSIONS

In this communication, a methodology for quantifying changes in in situ reductive dechlorination rates due to a chemical amendment is described and demonstrated. The methodology is significant because progress in the development of bioremediation technologies depends on an ability to measure the effectiveness of bioremediation techniques

in the field. To the best of our knowledge, this communication also describes the first use of fumarate in a field method designed to enhance reductive dechlorination rates. TCFE reductive dechlorination rates increased from 8.9 to 120 times in wells where reductive dechlorination was initially observed. Reductive dechlorination was stimulated in wells where it was not initially observed. Similarities in the transformation behaviors of TCFE and fumarate indicated that reductive dechlorination and fumarate reduction were supported by similar biogeochemical conditions.

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CHAPTER 5: SUMMARY

Methods for measuring in situ transformation rates are needed to assess the potential for natural attenuation and to evaluate engineered bioremediation approaches. However, in situ transformation rates are difficult to measure because both transformation and transport processes affect solute concentrations in groundwater. This dissertation describes the development and demonstration of a method for measuring in situ reductive dechlorination rates in TCE-contaminated groundwater using single-well, "push-pull" tests.

One novel component of the rate-determination method presented in this dissertation is the use of trichlorofluoroethene (TCFE) as a fluorinated surrogate for TCE. TCFE plays a critical role in the method because it makes it possible to interrogate a TCE-contaminated aquifer with an injected test solution. It would have been impractical to use TCE itself in the injected test solution because injected and background TCE are not distinguishable. TCFE was selected as a surrogate for TCE based on results from field experiments conducted in TCE-contaminated groundwater at the site of a former chemical manufacturing plant in the San Francisco Bay area. Results from field experiments indicated that TCFE and TCE experienced similar transport behavior in two distinct aquifer zones. Results from another set of field experiments indicated that TCFE underwent reductive dechlorination by a pathway analogous to that of TCE while retaining the fluorine label.

The second novel component of the rate-determination method presented in this dissertation is the "forced mass balance" (FMB) data analysis technique. Although a data analysis technique designed to determine in situ transformation rates for nonsorbing solutes from push-pull test data had been described previously, the sorptive behavior of TCFE in the selected aquifer made it impossible to interpret TCFE data with that technique. Hence, the development of the FMB technique was critical because it made it possible to measure in situ transformation rates of sorbing solutes, such as TCFE, from push-pull test data. The FMB technique was evaluated by quantifying errors in rates derived by applying FMB to push-pull test data generated by a numerical model. Results from the evaluation indicated that errors in rates derived using FMB increase as the test duration, groundwater velocity, and ratio of reactant to product retardation factor increase. In addition, errors in derived rates increase as the reaction rate constant and aquifer dispersivity decrease. However, the error analysis also

indicated that a TCFE reductive dechlorination rate measured at the selected site was underestimated relative to the true in situ rate by only 10%.

Finally, the utility of the rate-determination method was demonstrated by using it to quantify changes in in situ reductive dechlorination rates due to the chemical amendment, fumarate (*trans*-1,2-ethenedicarboxylate). TCFE transformation rates were measured before and after three consecutive additions of fumarate in five wells. In wells where TCFE reductive dechlorination was observed before fumarate additions, first-order rate constants increased by factors ranging from 8.9 to 120. On the other hand, TCFE reductive dechlorination was stimulated by fumarate additions in wells where it was not initially observed. To the author's best knowledge, this is the first time that fumarate was used in field experiments to enhance reductive dechlorination rates.

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